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Metabolic Effects of Testosterone Propionate in Addison's Disease[1, 2]

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WHEN TESTOSTERONE propionate is given intramuscularly to man there occurs regularly a depression in the urinary excretion of nitrogen, inorganic phosphorus, sulfate, sodium, potassium and chloride and a gain in body weight due chiefly to water retained in association with salts and protein. This pattern of response varies little over a wide range of subjects tested including hypogonad men (1, 2, 3, 4) and women (4), normal men (2, 5) and women (2), aged men (6, 7) and the immature boy (8). In Cushing's syndrome in women (9) and in the aged man with osteoporosis (7) calcium is retained with nitrogen and phosphorus. This may not be peculiar to these conditions, however, and more protracted study of other subjects may well demonstrate the same process. The rise in basal heat production with testosterone propionate has thus far been well seen only in eunuchoids (1, 3, 10) but the protracted treatment necessary to establish an indubitable change has not been undertaken in others. Creatine excretion, when substantial or when sustained by creatine ingestion, may be depressed by testosterone propionate in the eunuchoid (1, 4), in the pubertal boy (8), and in the hypogonad woman (4) but the creatine tolerance of young boys (11) and normal men (12) has been reported to be uninfluenced. With this exception the state of gonadal function or the age of the subject seems to make relatively little qualitative difference in those metabolic responses to testosterone propionate which have been examined in sufficient detail. Other glands of internal secretion, however, may conceivably be intermediaries in these reactions.

The striking analogies between the sodium and chloride-retaining properties of the sex hormones and of several steroids of the adrenal

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series (5, 13) at once suggest that testosterone may act through the adrenal cortex. The association of retention of nitrogen, inorganic phosphorus and potassium with that of sodium chloride and water in the testosterone effect, however, is unlike any known reaction to an adrenal steroid. The disposition of adrenal extracts and desoxycorticosterone to promote potassium diuresis at once comes to mind as an illustration. Such a substance as corticosterone may induce nitrogen loss, apparently in the course of promotion of gluconeogenesis (14). The effect of some chemically unknown adrenal agent however may more nearly approximate that of testosterone. The general body growth and enhanced muscular development of the childish victims of adrenal cortical hyperplasia correspond to those seen in boys with interstitial cell tumors of the testis and bespeak anabolic influences from the adrenal not unlike those exerted by testosterone. It is not known whether the normal adrenal secretes such an agent.

In this account we have attempted to assess the importance of the adrenal cortex as an intermediary organ in the metabolic response to testosterone propionate by study of a man and a woman with Addison's disease. The subjects were maintained in good condition with added salt and desoxycorticosterone acetate. While we have no autopsy proof of complete absence of effective adrenal tissue in these people, it is clear that these glands were too imperfect to sustain sufficient secretion in response to the usual physiologic stimuli playing upon them. This does not exclude a conceivable reaction of residual tissue to the administered androgen but creates a presumption against it.

METHODS

The subjects were studied in the metabolism unit of the hospital where they received constant diets of sufficient caloric value to maintain body weight on schedules of limited and fairly regular activity. Each subject received sodium chloride supplements and 1 to 1.25 mg. of desoxycorticosterone acetate in sesame oil, intramuscularly, daily. They were in good condition throughout the experiments. The methods of dietary control and the techniques of chemical analysis of urine and blood and measurements of respiratory metabolism were the same as in our previous work (2, 3, 4) and need not be repeated here.

PROTOCOLS

T. K., was a 39-year-old man, a patient of Doctors Martin Goldner and H. T. Ricketts who kindly permitted these studies. He was first seen in August, 1941, suffering from fatigue, loss of weight (20 lb.), difficulty in walking and recurrent attacks of nausea and vomiting for 2 years. His skin was diffusely darkened, with intensification about the genitalia. The gums and buccal mucosa were pigmented. His blood pressure was 100/74 mm. Hg. The serum sodium was 126 and 120 milli-equivalents per liter, chloride 90 m. eq. per liter, potassium 5.3 and 5.7 m. eq. per liter. Urea nitrogen was 25 and 30 mg. per cent, non-protein nitrogen 42 mg. per cent. Roentgen-ray examination showed no evidence of pulmonary tuberculosis and no evidence of calcification of the adrenal glands. The testes, phallus and prostate glands were somewhat small in size.

The Addison's disease was complicated by posterolateral sclerosis of the spinal cord with involvement of the lower extremities which were spastic with increased tendon reflexes, positive Babinski signs, ataxia and diminished vibration sense (Dr. R. B. Richter). Bilateral pes cavus was present. Roentgenograms showed multiple congenital anomalies of the lumbo-sacral region including failure of fusion of the posterior segments of *lumbar 5* and *sacral 1* with partial lumbariza-

TABLE 1. EFFECT OF TESTOSTERONE PROPIONATE, 25 MG. DAILY, ON SEVERAL BLOOD CONSTITUENTS OF A MAN (*T. K.*) AND WOMAN (*P. K.*) WITH ADDISON'S DISEASE

Day	Hematocrit vol.%	Blood urea N mg.%	Serum Na m.eq./liter	Serum Cl m.eq./liter	Serum K m.eq./liter
			T. K.		
1		15.0	136.0	101.0	4.75
4		14.6	135.7	100.6	3.75
6		15.1	138.8	100.3	4.35
11		11.7	143.8	104.7	4.20
13		9.3	140.4	102.3	4.60
20		13.8	142.5	101.8	4.20
			P. K.		
1	39	13.3	139.3	106.1	4.95
8	37	12.9	143.3	106.7	5.05
10	36	9.6	141.4	105.0	4.38
14	35	9.5	141.6	102.6	4.70
16	37	10.1	139.5	103.7	4.63
23		13.3	138.1	102.1	4.45

The androgen was given intramuscularly from *day 0* through *day 11* in *T. K.* and from *day 8* through *day 13* in *P. K.* Enumeration of days as given in figure 1 and ..

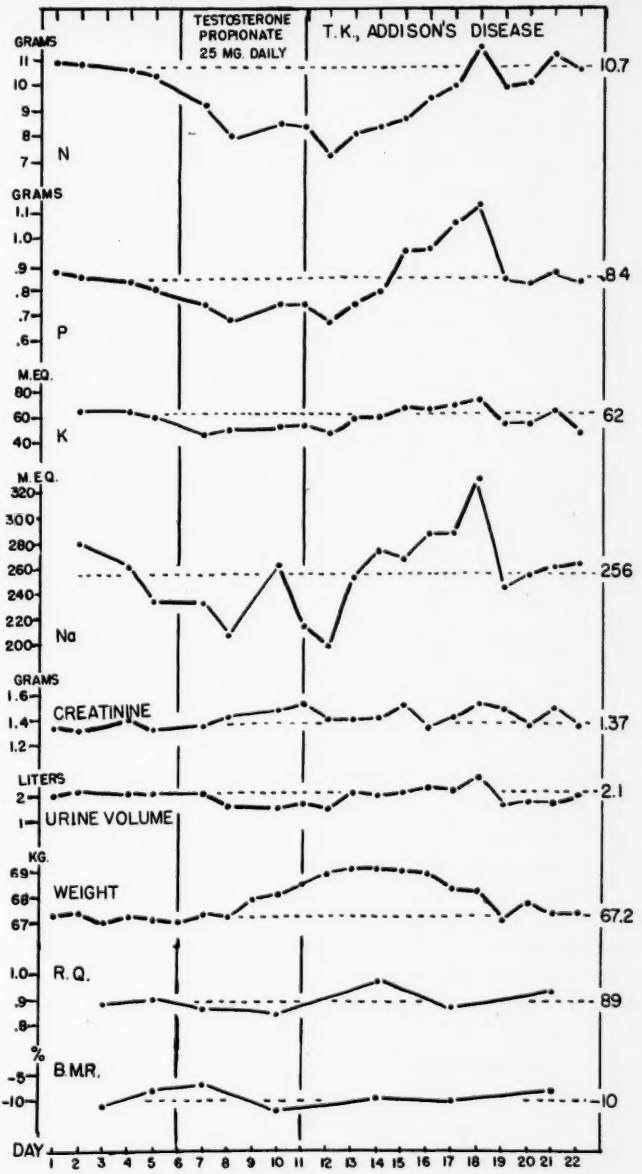

FIG. 1. Effect of testosterone propionate on several urinary constituents, body weight, respiratory quotient and basal metabolic rate of the man (*T. K.*) with Addison's disease. The subject was maintained in good condition with salt supplements and 1 mg. of desoxycorticosterone acetate daily. The dotted lines and the figures to the right thereof signify pretreatment baselines.

tion of the first sacral segment. Myelography with an opaque medium at a later date showed no lesion of the spinal canal. The spinal fluid was normal and Wassermann and Kahn tests on the serum were negative. There was no hematologic evidence of pernicious anemia and free acid was found in the stomach after administration of histamine.

In the interval between the first admission in August, 1941, and March, 1942, he was maintained in fair to good general condition on desoxycorticosterone acetate by injection with little or no supplementary salt. Toward the end of this period he took 2.5 mg. of desoxycorticosterone acetate daily and was troubled by occasional edema of the ankles.

In March, 1942, his response to testosterone propionate was studied. For this purpose he was placed upon a diet of C 247, P 71, F 112, Cal. 2280, yielding an estimated 11.4 gm. N, 1.76 gm. P, 0.96 gm. S, 1.50 gm. Na, 3.39 gm. K. To this was added 12 gm. of NaCl daily at the table. Fluid intake (2500 cc. daily), includ-

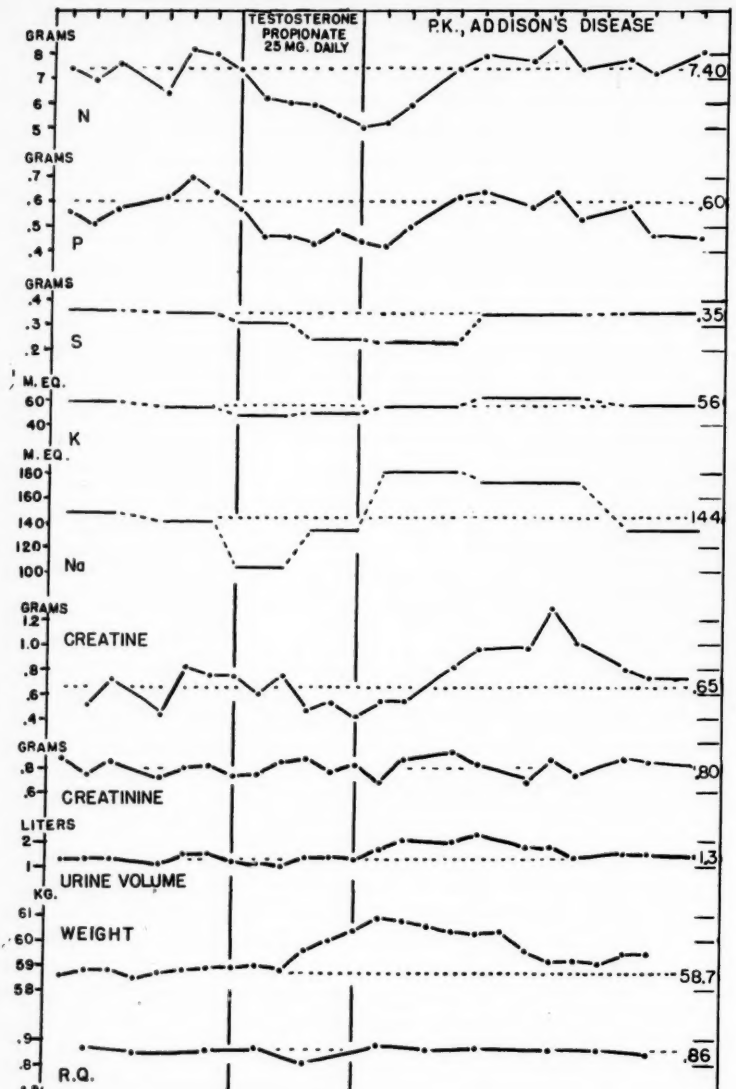

FIG. 2. Effect of testosterone propionate on several urinary constituents, body weight, respiratory quotient and basal metabolic rate of the woman (*P. K.*) with Addison's disease. The subject was maintained in good condition with salt supplements and 1.25 mg. of desoxycorticosterone acetate daily; 1.32 gm. of creatine hydrate was ingested daily. The dotted lines and the figures to the right thereof signify pretreatment baselines.

ing distilled drinking water, was kept constant in amount and composition. He received 1 mg. of desoxycorticosterone acetate daily intramuscularly. On this regimen he remained in good condition. His blood pressure ranged from 90/68 to 122/82, averaging in the neighborhood of 106/76 mm. Hg. The serum electrolytes and blood urea nitrogen, as seen in table 1 were normal. In the two weeks preceding treatment with testosterone propionate, 7 measurements of respiratory metabolism gave an average B.M.R. of −9 and an average basal heat production of 1539 Calories per day. Twenty-five mg. of testosterone propionate was given intramuscularly in sesame oil daily from April 16 through April 21. The data are recorded in figure 1 and table 1.

P. K. was a 39-year-old woman referred to us by Dr. E. C. Wrightsman. She was first seen in August,

1939, complaining of soreness of the tongue, recurrent vesicular lesions of the buccal mucosa for one year, darkening of the skin, faintness on arising in the morning, weakness for 3 months and loss of 19 lb. of weight in the preceding two months. She was deeply pigmented, there being a generalized browning of the skin with numerous smudges of deeper hue distributed over the face, neck, elbows, knees and backs of the hands with strong brown markings in the lines of the palms. There were numerous dark freckles especially on the forearms and sides of the neck. The mucosa of the lips was darkened and there were a few patches of pigment on the buccal mucosa and gum margins with darkish streaks on the tongue. The few aphthae on the buccal mucosa were not attributable to Addison's disease, nor was the history of the sore tongue. Her blood pressure was 116/58 mm. Hg, the systolic pressure declining 20 points on standing while the diastolic pressure remained unaffected. The serum sodium was then 137 m. eq. per liter, chloride 102, potassium 4.95. There was no elevation of the blood urea nitrogen. There were calcified lesions in the right apex, in the midportion of the right lung field and at the hila but no evidence of active tuberculosis. There was no evidence of calcification in the adrenal area. Menses were normal.

In the ensuing 3 years the patient has on the whole done well on moderate salt supplements and injections of desoxycorticosterone acetate. There have been bouts of nausea and vomiting and on one occasion, in association with a severe upper respiratory infection, she required emergency measures. On another occasion nausea, vomiting and collapse followed discontinuance of medication and an irregular schedule of living necessitated by the illness of her husband. Her blood pressure at one time reached 80/65 but has usually ranged about 110/72 mm. Hg. The serum sodium at one time was 128 m. eq. per liter but was usually normal. Previous to these studies she was taking 1.25 mg. of desoxycorticosterone acetate intramuscularly daily and about 3 gm. of supplementary salt.

In May, 1942, her response to testosterone propionate was studied. For this she was placed upon a diet of carbohydrate 238, protein 60, fat 94, calories 2039, yielding an estimated 9.6 gm. N, 1.16 gm. P, 0.77 gm. S, 3.30 gm. K, 0.82 gm. Na. To this 4 gm. of NaCl was added at the table and 6 gm. of NaCl given in a flavored drink. Her fluid intake including distilled drinking water was kept constant in amount (2100 cc. daily) and in composition; 1.32 gm. of creatine hydrate was given daily. She received 1.25 gm. of desoxycorticosterone daily, intramuscularly. She remained in excellent condition, the blood pressure ranging from 104/72 to 114/84 mm. Hg. The serum electrolytes as given in table 1 were normal. In the 2 weeks preceding treatment with testosterone propionate 6 measurements of respiratory metabolism gave an average B.M.R. of −7, and an average basal heat production of 1250 Calories. Twenty-five mg. of testosterone propionate was given daily from June 9 through June 14. The data are presented in figure 2 and table 1.

DISCUSSION

The pattern of response to testosterone propionate of the affected urinary constituents in a man (*T. K.*) and a woman (*P. K.*) with Addison's disease corresponds closely to that previously given for normal and hypogonad men and women. The depression in urinary nitrogen excretion begins promptly in both subjects, is well sustained for the duration of treatment and disappears slowly during the recovery phase of the experiment without conspicuous loss of the retained material in the time studied. The amount of nitrogen retained per kg. of original body weight per day at the time of sustained maximum effect is .043 gm. in the man and 0.39 gm. in the woman. This approximates values for normal young men and women (2) and aged men (6) and is somewhat less than the responses given by hypogonad men (2) and the one hypogonad woman studied (4). Urinary inorganic phosphorus declined sharply in both subjects during treatment. The discharge of the retained phosphorus during the recovery period in *T. K.* is not unique and was previously observed in an aged man (*M.L.*) with intact adrenals (6). A decline in urinary sulfate was seen in *P. K.* (fig. 2, table 2) during treatment. Urinary potassium declined in both subjects although the effects were more distinct in *T. K.* Although irregularities in urinary sodium excretion in *T. K.* somewhat mar the data, the conspicuous salt retention in both subjects under the influence of testosterone propionate was manifest and the loss of this retained salt was complete early in the recovery phase. This is the usual course of events in those with intact adrenals, diuresis occurring while vestiges of nitrogen retention remain. The salt and water retention further exemplified by the rapid weight gains is more prominent here than in other subjects. This difference may be accounted for by the higher salt intake of the patients with Addison's disease rather than by endocrinologic peculiarities. The spontaneous creatinuria of *T. K.* was too slight to permit definition of a testosterone effect and the data are not given. In *P. K.*, however, creatinuria was sustained at a high level by creatine ingestion and the usual depressing influence of the androgen was more distinct. In neither instance was creatinine excretion altered.

Basal heat production and the fasting respiratory quotients of the patients with Addison's disease were as little modified by testosterone propionate as in those with intact adrenals studied in experiments of this duration (2). It should be stressed once more that the calorigenic properties of testosterone propionate observed in the eunuchoid often require several weeks for unmistakable demonstration (3).

The concentrations of sodium, potassium and chloride in the serum of the patients with Addison's disease were unaffected by testosterone propionate (table 1) despite the retention of these minerals. No significant hemodilution as judged by hematocrit occurred in *P. K.* The depression of the concentration of urea nitrogen in both subjects is in all probability significant as it has been observed repeatedly before during treatment with this androgen. It is to be interpreted, we believe, as a reflection of the reduction in protein catabolism under the influence of testosterone propionate.

There is thus no reason adduced here for believing that testosterone propionate exerts any of its metabolic effects indirectly through the adrenal cortex. It is quite possible, of course, that adrenal cortical function may be necessary for testosterone response in the sense that tissue deprived of adrenal hormones may react poorly. Since our subjects were kept in good condition with added salt and small amounts of desoxycorticosterone acetate we can make no comments on this latter point and pertinent experiments would be difficult of execution in man. It may be recalled that several of the metabolic effects of testosterone propionate were likewise well manifested in a eunuchoid suffering from a supra-sellar cyst, shown at autopsy to have largely destroyed the pituitary gland (1), and in dwarfs with multiple physiologic evidence of hypopituitarism (15). The nitrogen-retaining action of methyl testosterone was demonstrated in dwarfs with hypopituitarism and both the calorigenic properties of this androgen and the enhancement of creatinuria were demonstrated in a cretin (16). Thus far no other gland of internal secretion has been shown to be an obligatory intermediary organ in metabolic reactions to androgens in man. Corroboration from animal experiments in which participation of remnant tissue can be more readily excluded will be awaited with interest.

SUMMARY

A man and a woman with Addison's disease, maintained in good condition by salt supplements and by daily injections of desoxycorticosterone acetate were given testosterone propionate intramuscularly, 25 mg. daily. The characteristic metabolic effects of the androgen including depression of urinary nitrogen, inorganic phosphorus, sulfate, sodium, potassium and creatine, with gains in body weight were well seen. The basal metabolic rates, fasting respiratory quotients and concentrations of serum electrolytes were unaltered within the time studied. No reason was adduced for supposing that the adrenal cortex is a necessary intermediary in the metabolic response to testosterone propionate in man.

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Addison's Disease in a Seven-Year-Old Boy[1]

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CASE REPORTS of classical Addison's disease in children are infrequent. Atkinson (1), reviewing the literature up to 1937, found 40 cases which he believed could be interpreted as adrenal insufficiency. Only 5 of these occurred in children under 10 years of age. Since that time, there have been 5 additional cases noted in the literature. Three of these have been classified in the syndrome of adrenal insufficiency associated with macrogenitosomia (Butler et. al., 2; Thelander and Cholffin, 3; Wilkins et. al., 4). Anderson (5) in 1942 reported the case of an 11-year-old boy with primary Addison's disease which he believed was the result of primary atrophy of the adrenals. Renshaw and Manning (6) observed a 12-year-old boy who, at autopsy, proved to have tuberculosis of the adrenals.

Recently we have had under observation a 7-year-old boy with adreno-cortical insufficiency, whose case report should perhaps be added to the growing literature on this subject. Although the etiology is not definite, it is felt that the picture presented by the patient may be the result of primary or post-infectious atrophy of the adrenal glands.

CASE REPORT

G. E., a 7-year-old white male, was admitted to the Communicable Disease Unit of the Los Angeles County Hospital on Aug. 11, 1942 with the complaint of stupor and sleepiness for 4 days.

Two and one-half months prior to admission, the patient's parents first noticed that his skin was becoming tan, apparently not related to sun exposure. Concomitantly, he developed marked enuresis, lost weight and fatigued readily at play. This condition continued until 5 days before his entry to the hospital when he began to vomit everything eaten and gradually became stuporous, for which he was brought to the hospital. On the day before admission he complained of headache and cramps in his legs.

The history revealed that when the patient was 2 years old he suffered a severe attack of 'pneumonia' which ran a course of 10 weeks, during which period he maintained a 'high fever' of from 4 to 5 weeks. At the height of his illness, numerous 'hemorrhages' of varying size appeared 'under' the skin and the boy 'bled from the mouth.' Subsequently, the hemorrhagic areas cleared, leaving a skin of normal appearance. Two years prior to his present admission, the patient had 'severe' chicken pox, with 'high fever' present for 5 weeks. There appeared to be no sequelae to either illness except frequent periodic attacks of vomiting, related to upper respiratory infections. At the age of 6, one year before coming under our observation, he had measles and pertussis, the latter lasting 6 weeks and being again aggravated by 'high fever.' The degree of fever in all instances was not known by the parents.

The patient was a full term normal infant, weighing 8 pounds at birth and delivered spontaneously. He was breast fed for the first 13 months, after which he was maintained on a regular feeding schedule and never became a feeding problem. Orange juice and cod liver oil were added to his diet early and in adequate dosage. At the age of 6 months he sat alone, while at the age of one year he began to walk. He did not talk in complete sentences until after the age of 2.5 years. His first tooth did not erupt until after the age of one year with the exact date not known. There is no history of immunization procedures.

As a younger child, the boy was always quiet and did not associate freely with other children. However, he was active and apparently enjoyed playing by himself. His appetite has always been good and his dietary habits have not been unusual except that during the last few years, and especially in the last year, he manifested a marked fondness for salted crackers, sauerkraut and dill pickles, preferring these to candy.

Enuresis had been present almost all of his life. This disturbance became more severe after the previously mentioned pneumonia and was especially severe

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[1] This patient was observed in the Los Angeles General Hospital Endocrine Clinic under the direction of Dr. E. Kost Shelton, Associate Professor of Medicine, University of Southern California, whose observations and comments were deeply appreciated by the authors.

before the present illness which precipitated his admission to the hospital.

The family history is not particularly informative. His parents, both 31 years of age, denied venereal disease, tuberculosis, epilepsy and all other significant diseases. Recent contacts with contagious disease and tuberculosis on the part of the patient were denied by both. Two siblings, a brother, age 9, and a sister, age 6 years, are living and well. The mother has had no other pregnancies.

FIG. 1. *G. E.*, age 7. Addison's disease. Note contrasts of color in nurse's skin and that of the patient.

On admission, the patient's temperature was 100.8°F. by rectum, pulse 138 per minute, respiration, 18, and blood pressure 60/30 mm. Hg. He was a thin, tall, markedly dehydrated white boy of the stated age of 7, in no acute distress but stuporous and apathetic. He lay quietly in bed and was co-operative. A deep brownish pigmentation of the skin was noted as being present over the entire body with an accentuation in the folds and creases. His pupils were regular and equal and reacted to light and in accommodation; there was no nystagmus and extra-ocular movements were normal. The tongue and buccal mucosa were dry. The gingiva and palatal mucosa presented small discrete areas of deep brownish-purple pigmentation. Passive movement of the neck revealed moderate stiffness in the last few degrees of anterior flexion. Results of examination of the heart and lungs were negative. The abdomen was scaphoid and there was moderate tenderness in the right lower quadrant which subsequently was not noticed. There were no palpable masses in the abdomen. The genitalia were normal in all respects except for slightly increased pigmentation. All deep reflexes were hypoactive but equal and the superficial reflexes were present and equal. There was a doubtful Brudzinski sign and a suggestive Kernig's sign bilaterally.

Initial laboratory work disclosed a 75 per cent hemoglobin (Sahli), 4,660,000 erythrocytes and 5,000 white blood cells with 70 per cent polymorphonuclear cells and 30 per cent mononuclears; abnormal forms were not identified. The urine analysis was negative for sugar, albumin and by microscopic examination. The blood Wassermann and Kahn reactions were negative. Spinal fluid examination resulted in a bloody tap with the 6 cc. of grossly bloody fluid containing 332 cells, 100 per cent of which were mononuclears. The pressure was under 50; Pandy test was two plus positive; and spinal chlorides were 650 mg. per cent. On culture no bacteria or acid-fast bacilli could be found. The blood chloride level at this time was 436 mg. per cent, sodium 320 mg. per cent, and potassium 32 mg. per cent. One week after admission, another spinal fluid examination revealed 10 cc. of xanthochromic fluid with a sediment containing many erythrocytes and an occasional lymphocyte. Pandy test was still two plus positive while the chlorides were now 670 mg. per cent. Culture for bacteria and acid-fast bacilli was again negative.

Two weeks after admission, the patient was transferred to the pediatric ward, service of Dr. Milo Brooks. There, with the boy asymptomatic and under treatment, a repeated spinal fluid examination was completely normal with the chlorides over 700 mg. per cent and a culture again negative for all bacteria, including acid-fast bacilli. At this time the blood sodium was 292 mg. per cent, icteric index 7, and albumin-globulin ratio 4.9–2.1. The basal metabolic rate was minus 27. A glucose tolerance test, by standard method, revealed the following levels expressed as mg. per cent: fasting, 80; first hour, lost; second hour, 100; third hour, 103; fourth hour, 100; and fifth hour, 69.

Roentgenograms of the chest on two occasions did not reveal any abnormal findings. Flat film of the abdomen revealed the renal contours to be within normal limits, the psoas shadows appeared normal, and there was no evidence of calcification in the suprarenal areas. A lateral film of the skull demonstrated a normal sella turcica with no evident intracranial pathological changes.

Ophthalmoscopic examination revealed normal fundi and refraction demonstrated normal vision. Punch biopsy of the skin demonstrated increased pigmentation of the basal cell layer.

At the time of admission, prompt relief from his symptoms followed the administration of adrenal cortical extract, intravenous salt and glucose. During his hospitalization the patient's course was characterized by frequent acute exacerbations of apathy, stupor, asthenia, nausea and vomiting, all associated with dis-

continuance of therapy as above. With the use of intramuscular adrenal cortical extract and adequate salt orally (parenterally in severe exacerbations), remissions invariably resulted with the boy becoming alert and manifesting a desire to play. No spontaneous remissions were noted. Evidence of meningeal irritation gradually cleared after his entrance to the hospital, and all such signs were absent on admission to the pediatric ward.

In view of the very strong probability that the patient was suffering from true Addison's disease, he was placed on a Cutler-Power-Wilder (7) regime with the diet as recommended and the withholding of all therapy for several days. However, on the day before the urine samples were to be collected, the boy lapsed into a severe crisis with profound asthenia and marked vomiting. Large doses of intravenous adrenal cortical extract (40 cc.), physiological saline solution, glucose and sodium citrate were necessary to evoke a remission at this time. Within 24 hours vomiting ceased and the patient's weakness and apathy had disappeared. In view of this crisis no further salt restriction tests were attempted. One week after this episode, with the patient receiving 10 cc. of adrenal cortical extract intramuscularly daily, and a diet high in glucose and salt, the blood chemistry revealed an NPN of 33, chlorides of 528, with sodium of 314 and potassium 28 mg. per cent.

Subsequently, the patient was given desoxycorticosterone, 5 mg. daily, but received this only 3 days, he being removed from the hospital at this time because of his family's urgent desire to return to their home in Kansas. On the day of discharge, blood studies disclosed an NPN of 31, chlorides of 500, with sodium of 302 and potassium of 19.1 mg. per cent. At this time the packed cell volume was 31 mm., plasma volume 69 and corrected sedimentation rate 3 mm. per hour. The hemoglobin was now 62 per cent; 3,340,000 erythrocytes, and 4,700 white blood cells with a differential count of 56 per cent lymphocytes, 32 per cent neutrophils, 12 per cent stabs, no monocytes, eosinophils or basophils. Reactions for the Mantoux test in concentration up to and including 0.1 mg. were all negative.

The boy's medication consisted primarily of the natural adrenal cortex hormone and later desoxycorticosterone, with a moderately high carbohydrate diet and additional sodium chloride orally, except on those occasions when crises demanded intravenous therapy.

DISCUSSION

The etiology of the Addisonian picture presented by this boy must remain hypothetical. Although it is generally conceded that the disease in the majority of such patients, both adults and children, is of tuberculous origin, this disease was never proven in the patient. The negative response to the Mantoux test on two occasions and the normal chest film mitigates heavily against such a diagnosis.

The early history, which we have emphasized, of 'hemorrhagic' complications during the pneumonia episode, and subsequent stormy illnesses of varicella and pertussis with high temperatures, offers a probable and ready explanation for adrenal atrophy. The adrenal gland is susceptible to degenerative, hemorrhagic or inflammatory changes, such as may have occurred in the above disorders. It is becoming more apparent that the incidence of atrophy of the adrenals is more frequent than has been generally accepted. Susman (8), for example, reviewed the literature and found 46 cases of atrophy in 189 patients with Addison's disease. In the individual series noted, the incidence of atrophy ranged from 10 to 50 per cent. Anderson's recent case report was offered as being one of adrenal atrophy. In our own patient, a hemorrhagic diathesis at the age of 2 may well have resulted in adrenal involvement with subsequent fibrosis and atrophy, while the severe illnsesses of later life may, in themselves, have initiated such a process or contributed to the already present degeneration. If is of interest that dietary inclinations toward salty food manifested themselves early in life. These may well have been concomitant with early adrenal involvement. Richter (9) has shown that there is a prompt sodium chloride craving in his adrenalectomized rats which is alleviated by administration of desoxycorticosterone.

The etiology of the meningeal irritation and spinal fluid changes remains obscure. Although at first it was felt that the probable explanation was tuberculous meningitis, the child's course and the subsequent laboratory findings did not substantiate such a premise. It is felt, in retrospect, that this acute meningeal episode bore no immediate relationship to the primary adrenal deficiency in the patient, and that a lack of adrenal cortical hormone probably did not produce the meningeal symptoms.

SUMMARY

A 7-year-old white boy with pigmentation, clinical findings and crises resembling those of Addison's disease is presented. An attempt to perform the Cutler-Power-Wilder excretion test resulted in severe crisis before the test could be completed. Clinical response to adreno-

cortical hormone and salt was prompt and adequate. The etiology remains obscure. It is hypothecated that adrenal damage occurring early in life from severe illness resulted in atrophy of these glands, However, tuberculous involvement has not definitely been disproven.

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Value of Desoxycorticosterone Acetate in the Treatment of Peripheral Vascular Diseases

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IN THE TREATMENT of thrombo-angiitis obliterans and of arteriosclerosis obliterans all will agree that successful therapy would encompass arrest of the disorder, maintenance of the circulation, and, if possible, increased circulation through the development of collateral vessels. Among the many methods and modalities of treatment advocated to achieve these results is the intravenous injection of a hypertonic solution of sodium chloride. A 2 to 5 per cent solution is injected 3 times weekly in amounts of 300 cc. or less, over long periods of time. Promotion of collateral circulation is the special forte of this treatment, which increases the volume and lessens the viscosity of the blood, effects which may be enhanced by the osmotic action of the sodium chloride itself.

Although no specific effect of this salt in thrombo-angiitis obliterans or in arteriosclerotic obliterans is claimed and although no deficiency in its ions has been shown to exist in these disorders, the remarkable benefits of salt therapy in many cases cannot be gainsaid. That salt metabolism is in some way intimately involved is apparent, and immediately invites speculation as to the possible rôle of the adrenal cortex which is concerned in regulating electrolyte metabolism. This gland becomes increasingly suspect when it is realized that the effect of its cortical hormone is to increase blood volume and lower blood viscosity. It is reasoned that a greater secretion of adrenal cortical hormone should increase the blood sodium chloride, increase blood volume, and lower blood viscosity and therefore should, within limits, have the same effect in thrombo-angiitis obliterans and arteriosclerosis obliterans as does intravenous hypertonic sodium chloride solution. It follows that the beneficial effects of sodium chloride solution, which benefits, theoretically, could be produced also by increased adrenal cortical activity may actually indicate an existing hypo-adrenia.

A comparison of the symptom complex of functional hypo-adrenia with the clinical picture presented in cases of thrombo-angiitis obliterans and arteriosclerosis obliterans, brings out a striking similarity. In common are the asthenia, muscle fatigue, sensitivity to cold, cold extremities, low rate of metabolism and hypotension. That, in these cases, a state of hypo-adrenia may exist suggests itself more and more, therefore, and would serve to explain further the beneficial effects of the salt solution. If deficient cortical activity does exist, then the hormone synthesized by Steiger and Reichstein, besides influencing the distribution of electrolytes and fluids, may also exert a specific effect. With this in mind, experimental use of desoxycorticosterone acetate in cases of thrombo-angiitis obliterans and arteriosclerosis obliterans seemed worthy of trial and an account of its use in 20 such cases is herein recorded.

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MATERIAL AND METHODS

The method followed was to prescribe desoxycorticosterone acetate in various unselected cases of thrombo-angiitis obliterans and of arteriosclerosis obliterans. With some patients the injection of the substance was the only form of treatment; the patients were allowed to continue smoking. The majority were patients who had had various forms of treatment previously, including intravenous saline, and it was felt that improvement, if any, specifically due to desoxycorticosterone acetate could be judged on a comparative basis. Subjective improvement was chiefly gauged by recording the number of blocks the patients could walk without pain, both before and after therapy. Clinical improvement was measured mainly by oscillometric and skin-temperature readings. The Pachon Boulitte oscillometer was used; a

McKesson Dermalor was employed in recording temperatures. Although room temperatures were not ideal, nevertheless by having each patient expose the feet for some time before the initial reading, with the room temperature approximately constant, any great discrepancy was overcome. The readings were spaced so that there was one-half hour of rest before the first reading, followed immediately by the injection; one-half hour later the second reading was taken. Even though no exhaustive study of method or procedure was attempted, a careful reading of the case reports will show that the treatment was given a fair trial and that the study was controlled as carefully as possible.

CASE REPORTS

Case 1. W. B., male; age, 41 years, a Gentile of German descent. The onset of symptoms occurred 17 years ago with coldness of both hands and feet, intermittent claudication and a history of migrating phlebitis. The left leg was amputated below the knee 14 years before. The right foot showed trophic changes with a scarred, bulbous right big toe and absence of lanugo. No pulse was palpable around the ankle and there was marked plantar ischemia. Oscillometric readings ranged from zero to a faint trace. The blood pressure was 140 mm. Hg systolic, 90 diastolic; the urine was sugar free. The blood Wassermann reaction was negative, the blood count within normal limits, blood chlorides 560 mg. per 100 cc. and roentgen-ray study of the legs showed no calcification of vessels.

The patient has been treated with saline 2 to 5 per cent and 300 cc., from once to 3 times weekly at various times for the past 10 years. Oscillometric readings showed that the main arterial tree was actually occluding in the right leg, but that a sufficient collateral circulation had developed for maintenance.

He was given desoxycorticosterone acetate, 10 mg. intramuscularly weekly, for a period of 6 months. He could walk 2 to 3 blocks without pain, initially, and this ability was maintained, although not improved upon. A drop in blood pressure was noted within ½ hour after a desoxycorticosterone acetate injection. Oscillometric readings showed an increase, ½ hour later, from a faint trace to a full 0.5. The patient's condition was maintained subjectively throughout this period with slight clinical improvement.

Case 2. A. B., male; age, 40 years. Diagnosis, arteriosclerosis obliterans. The intermittent claudication had been worse in the calf of the right leg for the past two months. He could walk about a mile without pain. He smoked occasionally. The feet did not feel cold. All vessels, except the left dorsalis pedis, were palpable around the ankles. There was bilateral plantar ischemia; the oscillometric readings of the left ankle were 4.5 and of the right ankle, 5.5. The blood Wassermann reaction was negative and the urine clear. Roentgen-ray examination of the legs showed calcification of the blood vessels.

Case 2. READINGS DURING PERIOD OF TREATMENT WITH DESOXYCORTICOSTERONE ACETATE

	Before therapy	0.5 hr. after
Blood pressure, mm. Hg	150/80	130/65
Skin temperature, °C., rt. toe/left toe	25.5/24.5	32.0/32.5
Oscillometric readings, rt. ankle/left ankle	7.0/8.5	8.0/8.5

The patient was given desoxycorticosterone acetate, 10 mg. intramuscularly, twice weekly. He repeatedly asserted that he felt much better after receiving the medication. He was able to walk as far as 2 miles at times without pain. He continued to smoke. The pulse of the left dorsalis pedis became palpable and oscillometric readings showed an increase. Of particular note in this case, in which readings were made ½ hour after an injection of desoxycorticosterone acetate, were: *a*) a fall in blood pressure, *b*) a rise in temperature readings of the skin of the feet, *c*) no change or slight improvement in oscillometric readings.

After 6 months of therapy the patient could walk indefinitely without pain. The oscillometric readings were maintained at a high level: right ankle, 8.0/left ankle, 8.0. The blood pressure reading showed a drop ½ hour after desoxycorticosterone acetate injections. The patient was grateful for what was being done for him. An increased collateral circulation need not have been achieved, but arterial spasm was overcome.

Case 3. E. H., male; age, 60 years. Diagnosis, arteriosclerosis obliterans. He complained for the past year of pain in the calf of the right leg after walking ½ to 2 blocks. He was not diabetic and smoked a pipe. On examination both feet were found to be cold. There were no palpable pulses around the ankles and there was plantar ischemia of the right foot on elevation. Oscillometric readings were: right ankle, very faint/left ankle, 2.0; roentgen-ray examination of the legs showed early sclerosis in both lower extremities. The blood Wassermann reaction was negative and blood chlorides were 456 mg. The skin temperature readings were: right toe, 19° C./left toe, 20° C.

The patient was given desoxycorticosterone acetate, 10 mg. twice weekly, as the sole form of treatment. Smoking was continued. The latter part of January, after 4 months of treatment, he said he felt much better and that he could walk a distance of 7 to 8 blocks without pain. The skin temperature readings were 24° C. in each foot. Recently he could walk as far as 10 blocks without pain; the pain when it came was not severe.

Case 3. READINGS DURING PERIOD OF TREATMENT WITH DESOXYCORTICOSTERONE ACETATE

	Before therapy	0.5 hr. after
Blood pressure, mm. Hg	185/105	150/90
Skin temperature, °C., rt. toe/left toe	26.5/25.0	25.2/26.5
Oscillometric readings, rt. ankle/left ankle	1.5/3.0	1.5–2.0/3.0

Case 4. A. U., female; age, 67 years. The patient had been a known diabetic for the past 2 years. For the preceding 4 weeks she had had pain in the left lower extremity when she walked as far as 1 to 2 blocks, and at night when in bed. The left foot was colder than the right. There was left plantar ischemia on elevation and the posterior tibial arteries were not palpable. Oscillometric readings were: right ankle, 0.5/left ankle, 0.5; skin temperature, right toe, 25° C./left toe, 25° C. The urine contained sugar (she attends the diabetic clinic). The blood Wassermann reaction was negative and skiagrams of the legs showed calcification of the vessels in both extremities.

Treatment with desoxycorticosterone acetate, 10 mg. weekly, produced no improvement. She is highly sensitive and emotional and this therapy was discontinued sooner than had been anticipated.

Case 5. H. J., male; age, 43 years. Diagnosis, arteriosclerosis obliterans. There was an onset of pain in the sole of the left foot, and intermittent claudication developed 4 months ago. The patient could walk a distance of 4 blocks without pain.

The general condition was good; the left foot colder than the right. There was left plantar ischemia. The pulse around the ankels was palpable with the exception of that of the left dorsalis pedis. Oscillometric readings were, right ankle, 3.5/left ankle, 0.25; skin temperature readings, right toe, 33° C./left toe, 32° C. The urine was clear; the Wassermann reaction negative; the blood chlorides were 475 mg. Skiagrams of the legs showed arteriosclerotic changes in the vessels. The blood pressure was 130 mm. Hg systolic, 80 diastolic.

The patient was given adrenal cortical extract 2 cc. intramuscularly twice weekly. There was subjective improvement, and he could walk as far as 10 blocks without pain. However, there was no clinical improvement. Oscillometric and temperature readings showed a decrease rather than an increase from the original readings.

Case 6. M. S., male; age, 41 years. Diagnosis, thrombo-angiitis obliterans. The patient gave a history of painful feet for the past 4 years for which he had been treated as due to flat-feet. For the past 6 months his condition had become much worse. There was no familial history of thrombo-angiitis obliterans or of migrating phlebitis. The hands were not affected. He smoked 20 cigarettes daily.

On examination rubor in the left foot and atrophy of the left leg were noted. The right foot was pale. No pulse was palpable around either ankle and there was bilateral plantar ischemia on elevation. Oscillometric readings were, right ankle, 0.0/left ankle, 0.0; skin temperature readings, right toe, 28° C./left toe, 29.5° C. The urine was clear; the Wassermann reaction negative. Skiagrams of the legs showed no evidence of sclerosis. The blood count was within normal limits. The electrocardiogram showed no evidence of cardiopathy; the blood pressure was 120 mm. Hg systolic, 80 diastolic. There was discoloration of the gum margins as in Addison's disease; however, there was neither asthenia, nausea nor vomiting.

The patient was first given desoxycorticosterone acetate, receiving 10 mg. intramuscularly twice weekly; he was instructed to take a teaspoonful of salt daily. This form of treatment was continued from Oct. 24, 1940 to Jan. 23, 1941. He showed no improvement whatsoever and as the case was far advanced, saline therapy was instituted. It is of interest to note that there has been no improvement with saline therapy up to this time. A return to treatment with desoxycorticosterone acetate produced subjective improvement. The clinical status, however, remained unchanged.

Case 7. J. R., male; age, 50 years. Diagnosis, arteriosclerosis obliterans. The onset of symptoms was 1 year ago with intermittent claudication of the calf of the right leg only; the patient could walk a distance of but 1 block without pain. He smoked 20 cigarettes daily. Both feet were cold. No pulse was palpable around the ankles and he had right plantar ischemia. Oscillometric readings were, right ankle, 0.0/left ankle, 2.5; skin temperature readings, right toe, 19° C./left toe, 21° C. Skiagrams of the legs showed early sclerosis of the vessels. The urine was clear, the Wassermann reaction negative and the blood chlorides 350 mg. per cent. Desoxycorticosterone acetate injected twice weekly intramuscularly for 6 months resulted in marked subjective and clinical improvement. The patient can walk about 10 blocks without pain and continues to smoke 10 cigarettes daily. Oscillometric and skin temperature readings improved and it is interesting to note that the blood pressure continued to fall, contrary to the general belief that the adrenal cortical hormone produces a rise in blood pressure in all cases.

Case 7. READINGS DURING PERIOD OF TREATMENT WITH DESOXYCORTICOSTERONE ACETATE

	Before therapy	0.5 hr. after
Blood pressure, mm. Hg	204/110	160/90
Skin temperature, °C., rt. toe/left toe	25/23	25.5/26.5
Oscillometric readings, rt. ankle/left ankle	0.0/3.0	0.0/4.0
Readings after cessation of therapy		
Blood pressure, mm. Hg	175/110	150/90
Skin temperature, °C., rt. toe/left toe	28/29.4	28.4/30.8
Oscillometric readings, rt. ankle/left ankle	0.0/5.5	0.0/5.5

Case 8. J. D., male; age, 47 years. Diagnosis, arteriosclerosis obliterans. The patient reported pain in both heels for the preceding 6 months after walking a distance of 2 to 3 blocks. He smoked 4 cigars daily. There was no history of migrating phlebitis. On examination all arteries around the ankle were palpable. There was bilateral coldness of the feet and bilateral plantar ischemia. Oscillometric readings were right ankle 2.5/left ankle, 2.0. The urine was sugar-free; the Wassermann reaction negative; the electrocardiogram, normal.

The patient was given 300 cc. of 3 per cent saline intravenously weekly from May 16, 1940 to Oct. 24, 1940, told to stop smoking and to take sitz baths following Buerger's exercises nightly. On this regime he did well. The symptoms markedly improved. He could walk as far as 2 miles without pain. The skin temperature readings were, right toe, 28° C./left toe 27° C. The blood pressure was 110 mm. Hg systolic, 70 diastolic.

From Oct. 24, 1940 to Jan. 23, 1941 he was given desoxycorticosterone acetate, 10 mg., intramuscularly weekly with 60 grains of sodium chloride daily by mouth. He claimed that the improvement had been maintained. He could still walk 2 miles without pain. After a further period of 4 months of this therapy he could walk without pain and considered himself very much improved. After therapy with desoxycorticosterone acetate the blood pressure was 110 mm. Hg systolic, 70 diastolic; the skin temperature readings were, right toe 27.4° C./left toe, 27.0° C. The oscillometric readings were, right ankle, 4.0/left ankle, 4.0.

Case 9. J. D., male; age, 43 years. Diagnosis, arteriosclerosis obliterans. The onset of symptoms had occurred 5 years ago with pain, chiefly of the left foot and ankle. He could walk a distance of but 1 block without pain. There was no history of migrating phlebitis nor a familial history of thrombo-angiitis obliterans. He smoked 3 packs of cigarettes per week.

On examination the left foot was found to be colder than the right. The right anterior tibial artery was palpable but no other vessels around the ankles were left. There was left plantar ischemia and the oscillometric readings were, right ankle, 2.0/left ankle, 0.75. The skin temperature readings were, right toe, 25° C./left toe, 25.5° C.

The urine was clear and the blood Wassermann reaction negative. A skiagram of the legs showed early calcification of vessels. He was given desoxycorticosterone acetate, 5 mg. weekly; later this was increased to 10 mg. twice weekly. In 2 months he could walk about one mile without pain. After 6 months he was able to walk several miles without pain and said he was a 'new man.' The oscillometric readings increased slightly in the right ankle but decreased in the left. Six months after the institution of treatment the readings were: blood pressure, 130 mm. Hg systolic, 80 diastolic; skin temperature, right toe, 29° C./left toe 29.5° C.; oscillometer, right ankle, 3.0/left ankle, 0.5.

Case 10. M. S., male. Symptoms and signs of arteriosclerosis were present. He complained of heaviness and weakness of both thighs, pain in both legs and feet, and general fatigue. Salicylates and tonics which had been given him in another clinic had not helped the condition. Examination of the legs revealed no trophic changes. The right foot was colder than the left; all pulses around the ankles were palpable, but there was slight left plantar ischemia. Oscillometric readings were, right ankle 0.5/left ankle, 0.5; temperature readings, right toe, 31° C./left toe, 32° C.

The urine was free of albumen and sugar; the blood Wassermann reaction was negative; an electrocardiogram revealed a normal cardiac status with a blood pressure of 135 mm. Hg systolic, 70 diastolic. A skiagram of the legs showed early sclerosis of the blood vessels. He was given 2 per cent saline intravenously, 300 cc., sitz baths and Buerger's exercises daily and not allowed to smoke. This treatment was maintained for many months with only slight improvement. Desoxycorticosterone acetate, 10 mg. intramuscularly was instituted in place of the saline. The patient improved generally, the fatigue lessened and he walked better, without pain and for longer distances. The blood pressure did not vary, the skin temperature readings remained about the same, but, contrary to most cases, the oscillometric readings increased from time to time without maintaining the upper levels, showing that spasm of the vessels had been temporarily relieved.

Case 11. B. M., male; age, 56 years. Diagnosis, arteriosclerosis obliterans. There was pain in the left thigh and calf on taking 5 steps; the onset occurred about 3 years ago. He smoked 10 cigarettes daily. On examination the feet felt cold and no pulse around the ankles was palpable. He had marked left plantar ischemia on elevation (Samuels' test) and oscillometric readings of right ankle 1.5/left ankle 0.0; skin temperature readings, right toe 20° C./left toe 20° C.

The urine gave a slight trace of albumen but no sugar. The blood Wassermann reaction was negative, the blood chlorides 410 mg. per 100 cc. and blood cholesterol 180 mg. Roentgen-ray examination showed moderate sclerosis of the arteries of both legs. The patient was told he could continue smoking and received 10 mg. of desoxycorticosterone acetate twice weekly while taking a teaspoonful of salt daily. After 7 months of therapy there was no marked subjective improvement. Clinically there was some evidence of improvement. The blood pressure was 140 mm. Hg systolic, 75 diastolic; oscillometric readings, right ankle, 1.5/left ankle, 0.0; skin temperature readings, right toe, 27.8° C./left toe 29.0° C. The increase in temperature readings would seem to indicate peripheral vasodilatation.

Case 12. J. K., male; age, 47 years; Jewish male of Polish extraction. Diagnosis, thrombo-angiitis obliterans. The onset of symptoms occurred in 1935 when, due to massive gangrene of the left foot, that leg was amputated. Since then there has been claudication pain in the calf of the right leg enabling him to walk only 1 to 2 blocks without stopping. The right foot was cold but showed no trophic changes. No pulse could be felt around the ankle and there was marked plantar ischemia on elevation. Oscillometric readings around the right ankle have varied from zero to 1.0 in the past few years. The roentgen-ray findings were negative; the urine was clear; the blood Wassermann reaction negative; the blood study showed 103 per cent hemoglobin, red blood cells 4,850,000, white blood cells 7,800; the differential count was normal.

He has remained under treatment for several years since the amputation, receiving the usual advice to

Case 13. READINGS DURING PERIOD OF TREATMENT WITH DESOXYCORTICOSTERONE ACETATE

	Before therapy	0.5 hr. after	Before therapy	0.5 hr. after	Before therapy	0.5 hr. after
Blood pressure, mm. Hg	116/70	110/70	105/70	105/65	105/60	105/70
Skin temperature °C., rt. toe/left toe			21.0/25.5	23.8/22.2	30.5/33.0	31/33.5
Oscillometric readings rt. ankle/left ankle	6.0/7.0	7.0/7.0			6.0/8.5	6.0/9.0

Case 14. READINGS DURING COURSE OF TREATMENT WITH DESOXYCORTICOSTERONE ACETATE

	Before therapy	0.5 hr. after	Before therapy	0.5 hr. after
Blood pressure, mm. Hg	120/75	110/70	105/70	105/75
Skin temperature °C., rt. toe/left toe	22.5/21.8	24.8/24.8	21/21	21.8/21.2
Oscillometric readings rt. ankle/left ankle	0.0/0.0	0.0/0.0	0.0/0.0	0.0/0.0

refrain from smoking; he has taken Buerger's exercise and sitz baths, and in addition, intravenous saline, 300 cc. varying from 2 to 5 per cent. He was also given tissue extract for a period of time but felt better when taking the saline. He had poor arm veins so that the external jugular veins were being used for the injections—not without some misgiving. He was given desoxycorticosterone acetate, 15 mg. intramuscularly 3 times weekly for several months. Skin temperature readings were not recorded but blood pressure and oscillometric readings were made frequently before and ½ hour after the desoxycorticosterone acetate injections. Following most of the injections there was a drop in blood pressure of 10 to 20 points. There was never a rise in blood pressure. Oscillometric readings showed no increase after the injections and ranged between 0.5 and 1.0 at the right ankle. Some days he felt better than other days and reported he could walk as much as 3 blocks without having to stop. He experienced thirst and a salty taste in his mouth after the injections, similar to that following saline therapy. No definite improvement was obvious but the patient maintained that he felt better at times, although not consistently so. The desoxycorticosterone acetate injections may have maintained his system in the same state as did the saline injections, but it is possible that some of his sense of well-being was psychological.

Case 13. J. H., age, 30 years, male, Gentile with a history and symptoms suggestive of thrombo-angiitis obliterans. The onset of symptoms occurred 8 years ago but the right foot has become really troublesome only recently. A history of migrating phlebitis was obtained. He could not walk more than 10 to 15 blocks without pain. He was a pipe smoker. On examination the feet had a good color, there were no trophic changes, the right foot felt colder than the left, and pulsations of the dorsalis pedis, anterior tibial and posterior tibial arteries, although felt in the left foot, could not be palpated in the right foot. Plantar ischemia on elevation could be discerned on the right foot. Oscillometric readings were, right ankle, 5.5/left ankle, 8.0. The heart findings were normal, the urine clear, blood Wassermann reaction negative and roentgen-ray study showed no calcification of blood vessels. Following therapy with intravenous saline he felt very much improved and could walk great distances. He was then given desoxycorticosterone acetate, 10 mg. intramuscularly twice weekly to see if this improvement could be maintained. During this trial period the condition did not regress but he was never quite as happy as when receiving saline therapy.

Case 14. M. M., male; age, 47 years. Diagnosis, arteriosclerosis. For the past 4 years he has had pain in the buttocks with claudication pain in the calves of legs after walking one block. In the past 2 years the symptoms have become progressively worse. He claimed to have stopped smoking in recent years. On examination of the lower extremities, there were no trophic changes; both feet were cold, the right more so than the left; both anterior tibial arteries were faintly palpable but neither the posterior tibial nor dorsalis pedis pulse was felt. He had bilateral plantar ischemia and the oscillometric readings were zero at either ankle. The urine was sugar-free but showed a slight trace of albumen; the blood Wassermann reaction was negative and roentgenograms of the legs revealed calcified plaques in the upper tibial arteries. Cardiac examination and electrocardiogram showed myocardial infarction with insufficiency and coronary closure.

He was given the usual instructions and received tissue extracts by intramuscular injections. Intravenous saline could not be used in this case because of the cardiac status. He obtained no relief with the tissue extracts so that desoxycorticosterone acetate, 10 mg. intramuscularly twice weekly, was substituted. During an 8 months' trial varied results were obtained. Blood pressure, temperature, and oscillometric readings were made at intervals. At times he felt improved, on which occasions there was a drop in blood pressure with a rise in temperature following treatment with desoxycorticosterone acetate.

For a period of two months he was given a combination of 5 mg. of desoxycorticosterone acetate and 0.5 cc. of tissue extract, intramuscularly. During this period it seemed that general improvement had been greater with desoxycorticosterone acetate therapy alone. It is to be noted that for a patient with this degree of artiosclerosis, the blood pressure can be considered low.

Case 15. I. L., male; age, 55 years. Diagnosis, arteriosclerosis obliterans. He has had diabetes for the past 8 years and presented himself complaining of claudication pain in the calf of the right leg after walking a distance of 2 to 3 blocks. The onset of symptoms

Case 15. READINGS DURING THE COURSE OF TREATMENT WITH DESOXYCORTICOSTERONE ACETATE

	Before therapy	0.5 hr. after	Before therapy	0.5 hr. after
Blood pressure, mm. Hg	154/80	154/80	150/85	155/85
Skin temperature, °C., rt. toe/left toe	33/33	33.2/33.2	32/32.8	32.6/33.4
Oscillometric readings, rt. ankle/left ankle	1.5/2.5	3.0/3.0	1.5/2.5	1.5/3.5

was 6 months earlier. He smoked between 1.5 and 2 packs of cigarettes per day. On physical examination, there were no trophic changes; the feet were of good color and the right dorsalis pedis and right and left anterior tibial arteries were palpable. Had had plantar ischemia in the right foot and the skin temperature readings in both the right and left toes were 33° C.

The oscillometric readings were, right ankle 1.5/left ankle 2.5. The urine analysis gave normal results, the blood Wassermann reaction was negative; roentgenograms of legs showed no calcification and electrocardiograms showed some myocardial damage. The blood chlorides were 538 mg.

The usual advice was given and treatment consisted of desoxycorticosterone acetate, 5 mg., 2 times weekly. Blood pressure, temperature readings and oscillometric readings were made from time to time both before and 0.5 hour after the desoxycorticosterone acetate injections. On only two occasions did a rise in blood pressure occur. At all other times there was a lowering or no change after the injections. The majority of the skin temperature readings showed a rise from .2 to 1.4° C. Oscillometric readings either remained the same or showed an increase.

Subjectively, he could walk longer distances without pain—as much as 5 or 6 blocks. However, he was never completely free from pain so that often he would come in saying he felt no better. In this case the readings showed clinical improvement when the patient complained he felt no better.

Case 16. A. K., male; age, 50 years. Diagnosis, arteriosclerosis obliterans. He has had trouble with his feet for about a year and a half and could walk but 4 to 5 blocks without pain. He also complained of burning and tingling of the feet. He smoked 40 cigarettes daily. There were no tropic changes of the feet and no varicosities. The feet felt warm to the touch. He had bilateral plantar ischemia which was more marked on the left foot. No peripheral pulse was palpable. Oscillometric readings were, right ankle, 0.5/left ankle, 1.0. The results of urine analysis were normal and the blood Wassermann reaction was negative. Roentgen-ray examination of the legs showed calcification of the blood vessels.

Besides the usual advice and home treatments he

Case 16. READINGS AT ONE TIME DURING COURSE OF TREATMENT WITH DESOXYCORTICOSTERONE ACETATE

	Before therapy	0.5 hr. after
Blood pressure, mm. Hg	125/70	130/90
Skin temperature, °C., rt. toe/left toe	29.4/29.4	31.5/31.4
Oscillometric readings, rt. ankle/left ankle	1.25/0.5	1.25/0.5

was given intravenous saline for several months, with no improvement resulting either clinically or subjectively. He was then given desoxycorticosterone acetate and he said that he felt somewhat improved.

Case 17. A. K., male; age, 58 years. He has had trouble with his feet for 12 years but first reported to the clinic 5 years ago. At that time he could walk a distance of 4 or 5 blocks without pain and there was no involvement of the upper extremities. After 3

Case 17. READINGS AT ONE TIME DURING THE PERIOD OF TREATMENT WITH DESOXYCORTICOSTERONE ACETATE

	Before therapy	0.5 hr. after
Blood pressure, mm. Hg	110/70	115/70
Skin temperature, °C., rt. toe/left toe	31.8/32.2	33.4/34.4
Oscillometric readings, rt. ankle/left ankle	0.0/0.5	0.0/0.5

months of treatment with diathermy and tissue extract he stopped attending the clinic, returning only when he could walk but one-half block without pain in the feet and calves of the legs. On physical examination there was no trophic changes; the pulse around the ankles was not felt and he had bilateral plantar ischemia. Oscillometric readings, right ankle, 0.0/left ankle, 1.0. The urine analysis gave negative results, the blood Wassermann reaction was negative and roentgen-ray examination showed no calcification of tibial vessels. An electrocardiogram revealed myocardial damage, probably with coronary involvement.

For 3 months he received mecholyl iontophoresis, diathermy and tissue extract but his condition progressively became worse. These treatments were stopped and he was given desoxycorticosterone acetate, 5 mg. intramuscularly twice weekly. For 3 months these treatments also failed to give relief. He was then given both desoxycorticosterone acetate and tissue extract. During a two-month trial of this therapy he felt improved. He no longer has pain while resting; he can walk longer distances and the pain is not as severe as previously. There are no marked changes in blood pressure, skin temperature, and oscillometric readings. For his age and general condition, the blood pressure can be considered low. In this case, a drop in blood pressure following the desoxycorticosterone acetate injections was never observed.

Case 18. H. S., male; age, 52 years. He originally complained of pain in the right foot and calf 8.5 years ago. He smoked about 20 cigarettes a day and gave no history of migrating phlebitis. A diagnosis of thromboangiitis obliterans was made at that time and he improved with saline therapy. He stopped treatment for about 8 years and recommenced smoking. He felt

well until recently when he complained of pain in the left foot and calf on walking. On examination the feet were of good color with no trophic changes or ulceration. The right dorsalis pedis artery was palpable but no other pulse around the ankles was felt. He had marked bilateral plantar ischemia. The skin temperature readings were, right toe, 27.2° C./left toe, 26.4° C. The oscillometric readings were, right ankle, 5.0/left ankle, 0.5. The urine was normal and the blood Wassermann reaction was negative. Roentgenograms showed no calcification of vessels.

Case 18. READINGS AT ONE TIME DURING THE PERIOD OF TREATMENT WITH DESOXYCORTICOSTERONE ACETATE

	Before therapy	0.5 hr. after
Blood pressure, mm. Hg	150/70	145/70
Skin temperature, °C., rt. toe/left toe	30.2/30.6	33.2/31.0
Oscillometric readings, rt. ankle/left ankle	5.0/0.25	4.5/0.5

He was given saline therapy but could walk a distance of only one block without pain. Therapy with desoxycorticosterone acetate, 5 mg. intramuscularly, three times weekly was then instituted. During this period he felt better. He could walk a distance of 3 to 5 blocks without pain.

There were no marked clinical changes, although the temperature readings steadily improved and remained at higher levels. The blood pressure varied and was as low as 120/75 mm. Hg.

Case 19. J. G., male; age, 65 years. Diagnosis, arteriosclerosis obliterans. For the past 4 months he has had pain in both feet and could walk only one-half block. The pain in the right foot was more severe than in the left. He smoked about 20 cigarettes daily. On physical examination, varicosities in both legs were found, but there were no trophic changes. The pulse of the left dorsalis pedis artery was palpable. The right foot was colder than the left with marked bilateral dorsal and plantar ischemia. The oscillometric readings were, right ankle, 0.0/left ankle, 1.0. The skin temperature readings were, right toe, 30.6° C./left toe, 32.8° C. The urine was normal as was the blood Wassermann reaction. Roentgenograms revealed no appreciable vascular calcification in either leg.

The patient was allowed to continue smoking while receiving desoxycorticosterone acetate injections, 5 mg. intramuscularly twice weekly. After a two-month trial he reported no subjective improvement, although he felt that his condition was getting no worse. The oscillometric readings at this time showed some increase; right ankle, 0.0/left ankle 1.5.

He was then given tissue extract. The condition is becoming progressively worse. He has to stop several times while walking one block.

Case 20. M. S., male; age, 62 years. Diagnosis arteriosclerosis obliterans. He complained of pain and coldness of the left foot for the past 5 months. The pain in the left foot came on after walking about 5 blocks. He smoked 7 or 8 cigarettes daily. He had no varicosities or trophic changes. The feet were cold, both left and right. No pulse was palpable around the ankles and there was left planter ischemia. The oscillometric readings were, right ankle, 6.0/left ankle, 0.0. There was a faint trace of albumen in the urine. The blood Wassermann reaction was negative. Roentgenograms of the legs showed no calcification and an electrocardiogram revealed no pathology. The blood pressure was 175 mm. Hg systolic, 80 diastolic.

Therapy with desoxycorticosterone acetate, 10 mg. intramuscularly for 3 months, produced no improvement in the symptoms. There developed, however, a positive oscillometric reading, right ankle, 6.0/left ankle, 1.0.

CONCLUSIONS

1. The beneficial physiologic effect of hypertonic solutions of sodium chloride intravenously in thrombo-angiitis obliterans and arteriosclerosis obliterans can, within limits, be attained by intramuscular injections of desoxycorticosterone acetate.

2. The desoxycorticosterone acetate often appears to lower blood pressure; more often it may cause an increase in local skin temperature and occasionally an increase in oscillometric readings. A permanent lowering in blood pressure was not obtained. There is no doubt that rest was a contributing factor in those cases in which the blood pressure was lowered.

3. Because results in 20 cases of peripheral vascular disorders have been encouraging more extensive clinical trials of desoxycorticosterone acetate should be undertaken.

4. Desoxycorticosterone acetate cannot be regarded as a substitute for intravenous saline therapy, but it may serve as an excellent adjuvant for those clinicians who favor this form of treatment. It is useful in those cases in which intravenous technic or sudden increases in blood volume are contraindicated.

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The desoxycorticosterone acetate (Doca) used in this study was supplied by Roche-Organon, Inc., Nutley, N. J. through the courtesy of Dr. M. C. James.

The case reports were obtained from Peripheral Arterial Disease Clinics, Fourth Division, Bellevue Hospital, New York City, and Stuyvesant Polyclinic, New York City; Dr. Saul S. Samuels, Chief.

Nutritional Therapy of Infertility in the Male, with Special Reference to the Vitamin B Complex and Vitamin E[1]

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IT HAS long been known that there is a relation between nutrition and fertility. Degenerative effects on the reproductive organs were among the most striking changes noted in early work on deficiencies of vitamin A, the vitamin B complex and vitamin E (1, 2, 3). Among these, the B vitamins have received less attention in this respect than the other two. Changes in the reproductive system in vitamin B deficiency have been variously attributed to an associated vitamin E deficiency and to the inanition which occurs in depletion of the B vitamins.

With the isolation, identification and synthesis of various factors of the B complex, reinvestigation of the early work has already yielded further information. Lepkovsky and Krause (4) found in the study of pantothenic acid deficiency that if control animals were restricted in food intake so that their body weight approximated that of animals deficient in pantothenic acid, the percentage difference in the weight of the testes in the two groups exceeded several-fold that in body weight or food intake.

Among a group of male patients examined by us during the past year because of sterility, we were impressed by the presence, in several, of lesions indicative of more or less severe vitamin B deficiency such as glossitis, stomatitis and lesions of the skin. These men complained of headaches, irritability, insomnia, mental depression, easy fatigability and lumbar backache. Although all but one appeared well-nourished, they gave histories of subsistence on grossly inadequate diets for many years.

We were led to attempt nutritional therapy in these patients not only because of the known relation of nutrition and fertility but also because of the experimental finding by one of us (M. S. B.) that the liver loses its ability to inactivate estrogen in deficiency of the vitamin B complex (5, 6, 7). In contrast, the inactivation of androgen in the liver was not significantly affected in B avitaminosis (8). The excess of free estrogen and the alteration in the estrogen-androgen equilibrium which thus result might be expected to affect adversely spermatogenesis as well as other functions of the endocrine system.

In male patients with cirrhosis of the liver, now known to result from nutritional deficiency, Glass, Edmondson and Soll (9) have shown that there is a considerable excess of estrogen in the urine and that all, or almost all of it appears in active, uncombined form; the amount of androgen in the urine was less than that of normal control males, and none of it appeared in free form. The patients studied by Glass and his associates had testicular atrophy, gynecomastia or both.

None of the patients included in the group to be reported here had gynecomastia, but two of them had definite testicular atrophy (excluding one case in which unilateral atrophy

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[1] Aided by a grant to one of us (M. S. B.) from the Winthrop Chemical Company, Inc.

followed an operation for varicocele). The occurrence of testicular atrophy in nutritional deficiency was reported by Reynolds and Macomber (10) more than 20 years ago.

For the most part, nutritional therapy in our patients was concerned with the vitamin B complex. A few patients received added supplements of vitamins A, D and C. A few that did not respond to B complex alone received synthetic vitamin E in addition.[2]

When the diet was grossly inadequate, instructions were given as to its correction, requiring inclusion of whole grains, fresh vegetables, fruits, dairy products, liver and meat, with limitation of intake of refined carbohydrates. In contrast to ingestion of the vitamin supplements, which, being 'medicine' were taken religiously, the dietary instructions appear to have been observed rather casually.

In 6 patients, examinations of the spermatic fluid were made biweekly or at monthly intervals during treatment. In others, when this was not possible, a specimen was examined before treatment was instituted and, in 2 cases, again shortly before impregnation occurred; in another case the second examination was made at the same time as the Friedman test, which proved positive. In 3 other cases in which impregnation occurred, only the initial sperm counts are as yet available.

Following initial success in the treatment of patients with obvious signs of nutritional deficiency, this therapy was extended to others living on a so-called 'normal' diet, who showed no noteworthy objective signs of impaired nutrition. Response to therapy was even better than in the original group; it seems not improbable that these apparently normal individuals had a low-grade nutritional deficiency.

CASE REPORTS

Case 1, (*J. K.*), age 39, had a 13-year-old daughter by a previous marriage. He had been married to his second wife (age 29) about 8 months when he was first seen early in September, 1941. At that time he had a sperm count of 20,000,000 per cc.; total 44,000,000; motile forms, 50 per cent; abnormal forms, 45 per cent. Previous examination of the wife revealed no abnormalities except for the possibility of a small fibroid in the right uterine cornu. The tubes, at first closed at a pressure of 200 mm. Hg, 10 weeks later were open at a pressure of 130 mm.

J. K., when seen in October, 1941, appeared apprehensive and depressed; he gave a history of a severe emotional disturbance following the death of his first wife several years previously. Formerly abstemious, he began to drink, neglected his diet and lost considerable weight. Subsequent to his second marriage his consumption of alcohol became more moderate and he paid more attention to his diet, although this was still deficient, partly due to use of a bland diet prescribed several years previously for a 'chronic appendicitis'; he no longer complained of abdominal pain.

Examination revealed a somewhat undernourished man 69.25 inches in height, 140 pounds in weight. He had a lateral nystagmus which had been present for 15 years, bilateral astigmatism, not completely corrected; the conjunctivae were severely congested. There was a small area of dermatitis about the size of a dime on the left side of the upper lip. This had been present for 5 years and had resisted repeated therapy with local applications and roentgen irradiation. The tongue was markedly edematous and showed deep indentations from contact with the teeth; there were numerous deep, red fissures which were very sensitive, and the papillae elsewhere were moderately atrophic. The teeth were in very poor condition; many had been extracted. The patient had an upper plate but, owing to tenderness of the gums, he had been unable to wear it for the preceding two months. There were numerous small ulcers on the inside of the lower lip.

The prostate was somewhat boggy but not enlarged; the testicles were soft and definitely smaller than normal. The urine contained a large amount of ether-soluble red pigment but was otherwise normal.

The patient was advised to take 2 heaping teaspoonfuls of brewer's yeast 3 times a day with meals and was given, in addition, 20,000 units of vitamin A, 2000 units of vitamin D and 50 mg. of ascorbic acid per day. When seen 2 weeks later he had gained 4 lb.; he was unable to take a full dose of the yeast because of flatulence and had reduced it to from 1 to 2 teaspoonfuls a day. The source of B complex was changed to 6 capsules a day of a fortified B complex preparation derived from liver, providing 6 mg. of thiamin, about 3.6 mg. of riboflavin and 60 mg. of niacin per day. A specimen of urine examined 2 weeks later contained no ether-soluble red pigment.

The patient was seen 3 days later, after slightly more than a month of treatment. The lesion on the upper lip had healed completely for the first time since it had appeared 5 years previously; he was much less apprehensive, reported that he felt much better generally, was less troubled by eyestrain and could now wear his dental plate. The tongue was less sensitive but showed little objective improvement. The ulcers on the lower lip had healed.

[2] Care was taken that the vitamin E was administered between meals on an empty stomach, to avoid possible destruction of the α-tocopherol through contact with rancid fat in the digestive tract; rancidity which might be developed in fats through processes ordinarily used in cooking is highly destructive to vitamin E.

Unfortunately it was not possible to check the sperm count of this patient subsequently. His wife was seen in April, 1942, at which time she was pregnant; her last period had occurred on February 1.

Case 2, (*N. W.*), age 27, had been married for 6 years. Precautions had been taken against pregnancy for the first 5 years of this time. Examination of his wife, age 26, showed no abnormalities.

A sperm count on *N. W.* in December, 1941, showed: 14,000,000 per cc.; total, 84,000,000; motile forms, 30 per cent; abnormal forms, 36 per cent. This patient had had severe acne on the face, chest and back since he was 15 years of age. He had received roentgen therapy to these regions with improvement in the acne; there were still a few pustules on the face, which was deeply scarred from the healed lesions. Three years previously he had been operated upon for a left varicocele. After the operation the left testicle atrophied. He had been sensitive to ragweed and had hay fever, not relieved by attempts at desensitization. Aside from the hay fever, he had frequent attacks of rhinitis throughout the year. His diet, while not adequate, did not appear to be seriously deficient.

Physical examination of *N. W.* revealed no abnormalities except for the acne, already mentioned, and a left testicle about $2 \times 1 \times 0.5$ cm. The right testicle appeared normal. The urine contained a large amount of ether-soluble red pigment.

A fortified liquid preparation of vitamin B complex derived from liver was prescribed in a dose of 2 teaspoonfuls 3 times a day; this provided per day: thiamin 9 mg., riboflavin 3.6 to 4.8 mg., niacin amide 60 mg. This was later increased to 8 teaspoonfuls a day, and from 50 to 75 mg. niacin amide in addition. After 2 months of therapy the ether-soluble red pigment in the urine had diminished to a trace; the sperm count was 18,400,000 per cc.; total, 154,560,000; motile forms, 75 per cent; abnormal forms, 22 per cent. At this time *N. W.*'s wife was pregnant.

Case 3, (*J. S.*), age 31, when seen in February, 1942, had been married for more than 3 years. Precautions against pregnancy had been taken for the first 2 years of this time. His wife, age 28, showed no abnormalities except a slight enlargement of the right ovary; the fallopian tubes were patent at a pressure of 60 mm. Hg.

A sperm count on *J. S.* on Feb. 10, 1942, showed 190,000,000 per cc.; total, 490,000,000; abnormal forms, 23 per cent. Only 1 or 2 sperm were motile in each high power field. The past history was essentially irrelevant, except that the patient reported a noticeable increase in fatigability during the preceding 2 or 3 years, associated with lumbar backache and pain in the calves of the legs. On physical examination the only positive finding was a moderate but definite atrophy along the margins of the tongue. For 4 months *J. S.* received 6 capsules a day of a fortified vitamin B complex preparation derived from liver, together with supplements of 150 mg. of niacin amide, 20,000 units of vitamin A, 2000 units of vitamin D and 50 mg. of ascorbic acid. At the end of this time the sperm count was 178,000,000 per cc.; total, 645,000,000; abnormal forms, 14 per cent. But still only 5 per cent were motile. The patient reported that he felt much stronger physically and was of a more cheerful disposition. He had recently played tennis for 2 hours at a stretch without undue fatigue; he no longer had backache or pain in the legs. The glossitis had improved.

Because of the continued necrospermia, α-tocopherol acetate was given in 3-mg. doses 3 times a day, on arising, and from 1 to 2 hours before meals.[2] After 6 weeks, the seminal fluid, while unchanged in other respects, had 41 per cent of actively motile forms. Five weeks later this had risen to 68 per cent. At this time the number of abnormal forms had diminished to 12 per cent. The progressive increase in motility in this case was very striking.

Case 4, (*W. L.*), age 28, had been married for 4 years. No precautions had been taken against pregnancy. His wife, age 23, showed no pelvic abnormalities; the fallopian tubes were patent.

On Oct. 28, 1941, a sperm count on *W. L.* showed only 2,100,000 in the whole specimen (0.9 cc.); 70 per cent were motile, 35 per cent abnormal. This patient gave a history of a seriously deficient diet; the only vegetable he ate was potato, the only fruit, a small amount of orange juice. He had otherwise subsisted for years on meat, eggs and pastries. He complained of severe migrainous headaches which were invariable on Sundays and holidays when he disturbed his usual routine. He had continuous lumbar backache, he was easily fatigued. During the prededing few months he had noticed a definite diminution in libido.

Physical examination revealed a moderate edema of the tongue, which showed deep indentations at areas of contact with the teeth, but there was no actual glossitis. The gums were soft, spongy, and bled easily; the teeth were in poor condition. Both testicles were soft and definitely atrophic.

For a period of 7 months this patient received 6 teaspoonfuls per day of a vitamin B complex preparation derived from liver, with supplements of niacin amide, 50 to 150 mg. per day, and vitamins A, D and C as indicated in *case 3*. Within a month the headaches and lumbar pain disappeared and have not recurred. There was marked improvement in general health and well-being and increased libido. However, repeated examinations of the spermatic fluid showed only moderate improvement. On June 8, 1942, the count was 9,200,000 per cc.; total, 44,160,000; 64 per cent motile; 31 per cent abnormal.

Alpha-tocopherol acetate was then given as in *case 3*. In 6 weeks the count was 31,500,000 per cc.; total, 94,500,000, motile forms, 90 per cent; abnormal forms, 25 per cent. Two months later, the count was 41,700,000 per cc.; total, 200,160,000—a several-fold increase.

In 5 other cases impregnation occurred after variable periods of therapy with vitamin B

complex alone.[3] In 2, only the initial sperm count is as yet available. In the other 3, in which the second count was made shortly before or shortly after impregnation, there was no significant change in the number, physical character or motility of the sperm. In one of the latter cases abortion occurred at about the fourth month of pregnancy.

In another case, for an account of which we are indebted to Dr. Edward A. Horowitz, sterility had persisted for 15 years. This patient, at our suggestion, received vitamin B complex and vitamin E and was instructed as to his diet. Within two months impregnation occurred.

Three further cases complete this series. In two of these, definite signs of nutritional deficiency were present. Under therapy with vitamin B complex alone both showed physical improvement together with an increase in the number of sperm and definite diminution in the percentage of abnormal forms. Impregnation had not occurred after 3.5 and 4.5 months of treatment, respectively, and both patients are now receiving α-tocopherol acetate[4] in addition.

The third patient had almost complete azoospermia; after centrifugation only one or two sperm could be found during prolonged search in an occasional specimen, although the sperm that were found were extremely active. This patient had a history of diphtheria in childhood, followed by months of complete disability. Since a few sperm could be found it was thought worthwhile to try vitamin B complex therapy; after 4.5 months there was no detectable change in the spermatic fluid.

DISCUSSION

It has been shown by Hotchkiss (11) that fairly wide variations occur spontaneously in semen specimens obtained from the same subject at different times. It may well be questioned, therefore, whether the changes observed by us may properly be ascribed to the treatment employed. In 5 of our cases, progressive improvement was noted in specimens examined at repeated intervals; it is unlikely that spontaneous variations would occur mainly in one direction and Hotchkiss (11) has further noted that men with relatively low sperm counts did not subsequently submit specimens that ranged into the higher levels and that the morphology in all specimens remained relatively constant. Among changes noted by us during therapy were, for example, an increase in the sperm count from 2,100,000 in the whole specimen to 41,700,000 per cc. and 200,160,000 in the whole specimen; a change in the percentage of motile forms from a small fraction of 1 per cent to 68 per cent; and diminution in the number of abnormal forms from 36 to 22, 23 to 12, 36 to 10 and from 38 to 28 per cent. Huffman (12) has noted an inverse proportion between the number of sperm per unit volume and the percentage of abnormal forms.

The observations of Hamblen (13) and of Hotchkiss (14) indicate that occasionally a man with a comparatively low sperm count and a high percentage of abnormal forms may be fertile. In one of our cases impregnation resulted when the count was 18,400,000 per cc. (total 154,560,000) and the percentage of abnormal forms was 22. In another, the count was only 10,100,000 per cc. (total 36,360,000) with 27 per cent abnormal forms (this is the case, already mentioned, in which an abortion subsequently occurred).

Conversely, it is well known that motile sperm of apparently normal morphology are not necessarily fertile. This is illustrated in 4 of our subjects in whom there were initially 72,000,000, 68,000,000, 170,000,000 and 144,000,00 sperm per cc., respectively, of which 70 to 90 per cent were motile. No significant morphologic or other visible changes occurred during therapy, yet fertility was apparently restored.

In general, in the patients in whom fertility was restored in this series, the percentage of abnormal forms fell within the ranges suggested by Moench (15) as indicating the likelihood of fertility. In only one patient did the percentage of abnormal forms exceed the

[3] Among the preparations of vitamin B complex that have been used in this study are: Vitamin B complex, Lederle Laboratories, New York City, oral and parenteral; Blexin, International Vitamin Corporation, New York City; Natuplex and Vitamin B complex, E. R. Squibb & Sons, New Brunswick, N. J.; Beminal, Ayerst, McKenna & Harrison, Ltd., Montreal, Que., and Rouses Point, N. Y. Beta plexin, Winthrop Chemical Co., New York City.

[4] The vitamin E preparation used in these cases was Ephynal Acetate, Hoffmann La Roche, Inc. Nutley, N. J.

figure associated by Moench with the usual presence of sterility (25 per cent). As already mentioned, the wife of this patient aborted.

Belding (16) believes that the total sperm count is a more accurate index of fertility than is the number of sperm per unit volume, since variable dilution of the sperm undoubtedly occurs. On this basis our results appear more striking than indicated in the case reports; the total counts usually increased by a greater percentage than the number of sperm per cc. In addition to the data already mentioned, in one case in which the count per cc. varied between 22,700,000 and 39,700,000 during treatment, the total count increased, with fluctuations from time to time, from 24,000,000 to 119,100,000 with a peak at 140,000,000.

One of our patients had previously been treated elsewhere with a variety of gonadotropic and androgenic preparations, without the slightest response. Subsequent vitamin therapy led to prompt and definite objective improvement in the number, motility and morphology of the sperm.

The observations we have made thus far indicate that deficient spermatogenesis may be related to faulty nutrition, perhaps far more often than hitherto suspected. A review of some of the nutritional deficiencies of the so-called 'normal' modern American diet has recently been published by the Council on Foods and Nutrition of the American Medical Association (17). Because the extent of nutritional deficiency in the United States is only beginning to be appreciated, the relationship of dietary inadequacy to endocrine dysfunction has received little attention. Only 8 years ago, Meaker (18) indicated that nutritional deficiency was of little significance for human sterility, 'except as regards the matter of insufficient protein.' And this belief was reiterated by Kreutzmann (19) two years ago. In the last few years, however, the publications of Sebrell, Spies, Sydenstricker, Jolliffe and others have emphasized the extent of nutritional deficiency, especially of the B vitamins, among all strata of the American population.

That the vitamins are not the only nutritional factors of possible clinical importance in spermatogenesis is indicated in a recent study by Holt and his collaborators (20) on the deleterious effects of deficiency of certain amino acids, especially arginine, on production of sperm in human subjects. In the treatment of these patients it is important to take into consideration the frequent occurrence of multiple nutritional deficiencies.

One of the problems raised in this study is the question of response to vitamin E. Deficiency in α-tocopherol in rats leads to irreversible changes in the testicle. As Mason (1) has pointed out, 'Even when vitamin E therapy is instituted coincident with the earliest appearance of histologic injury, the degenerative process continues to completion in all, or in the majority, of the seminiferous tubules. . . .' It remains to be determined whether the responses we have observed when vitamin E was given together with the B complex, after failure of the B complex alone to influence the sperm count sufficiently, constitute a real effect of vitamin E or a coincidental delayed effect of the B complex. The latter, in view of the time relationships, seems unlikely. But, with the patients in whom a control period of treatment with B complex preceded administration of vitamin E, no impregnation has yet occurred, despite improved spermatogenesis.

The relation of nutritional deficiency to the occurrence of sterility in the female is a problem which also requires study. For the most part, the wives of the patients in the series reported here ate substantially the same diet as did their husbands, and, as a consequence, their fertility may well have been impaired even though objective signs of deficiency were seen in only one of these women. Because of larger stature and greater energy output, men generally develop signs of deficiency more quickly and are likely to be more severely affected, than are women on the same diet. In a number of cases now under treatment both husband and wife are receiving nutritional therapy.

SUMMARY

Nutritional therapy, especially with the vitamin B complex, alone or in combination with vitamin E, was followed by improvement in the number, motility and morphology of the sperm in previously infertile men. In other cases of sterility in which initial specimens of spermatic

fluid were apparently normal, therapy with vitamin B complex alone apparently restored fertility without producing significant detectable changes in the sperm. Excluding one case of virtual azoöspermia, among 12 cases so treated 8 impregnations occurred. The wife of one patient aborted at about the fourth month; the remainder, at the time of writing, are approaching term or have been delivered of normal babies.

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Serum Diastase and Its Relation to Estrogen Metabolism in Pregnancy and the Menstrual Cycle[1]

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THE DETERMINATION of diastatic activity in the blood and urine is performed extensively as a diagnostic aid in suspected disease of the pancreas, high values being characteristic of acute pancreatic edema. Although the externally secreting cells of the pancreas have been considered by some as the source of diastatic activity, the presence of this enzyme in the blood after pancreatectomy has not been satisfactorily explained. Low levels have been reported in diseases associated with liver damage (1–5), but no one has been able to isolate the enzyme from the liver cell. Somogyi (1), in a recent review, concludes that its source is, as yet, unknown. Most investigators agree that under normal conditions its level in the blood is fairly constant, being unaffected by age, sex or diet.

Cope, et al., (6–8), working chiefly with dogs, have concluded that the internal secretions of all glands which are intimately related to carbohydrate metabolism influence serum diastase. In the dog, for example, they demonstrated sustained increases following hypophysectomy and adrenalectomy, and found the level of this enzyme a more sensitive index of adrenal insufficiency than changes in the nitrogenous or electrolytic substances of the blood.

In this laboratory blood and urinary studies of women during pregnancy and menstrual cycles have been concerned almost exclusively with the metabolism of the placental and ovarian hormones (9–15). That an intimate interrelationship between the various endocrine systems exists is well recognized although not fully defined. With the hope of throwing further light on this problem, especially the interrelationship between metabolism of the female sex hormone and those glands of internal secretion more directly involved in carbohydrate metabolism, it was decided to study the diastatic activity of the blood during periods of pregnancy and the menstrual cycle in which characteristic changes in estrogen and progestin metabolism have been established.

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METHODS

Diastatic activity was measured by the method of Scharles and Salter (16) as modified by Cope, et al. (17), for its determination in serum. By this method, glycogen loss is measured rather than the endproduct of glycogenolysis. Results, therefore, indicate the number of mg. of glycogen lost after a 3-hour period of digestion at 43° C. of 2 cc. of solution buffered at pH 7.0, containing 1 per cent of glycogen. All determinations were made in duplicate and are based on the activity of a 10 per cent serum concentration.

Glycogen was prepared from rabbit liver by the method of Sahyun and Alsberg (18).

EXPERIMENTAL RESULTS

Normal late pregnancy, labor and the puerperium. Specimens of blood were obtained repeatedly from 5 pregnant women during the last trimester and the puerperium. In 3 cases blood was also taken during active labor. Three of the 5 had diabetes, in all of whom the disease was being controlled by insulin and diet

and in whom, as in the other 2 cases, the pregnancy was proceeding normally. The results are presented in figure 1. In all 5 cases the concentration of blood diastase exhibited a tendency to increase from the 30th week on. In 2 of them a marked peak was reached at 38½ and at 39½ weeks, respectively, Unfortunately, no blood was obtained at this period from the 3 cases of diabetic pregnancies. In the 2 patients, one a diabetic, from whom blood was taken within 3 days of the onset of labor, a decrease in blood diastase was found and in the 3 cases from whom specimens were taken during labor an even more marked drop was apparent. With *E. H.*, 3 analyses were performed during labor. The result of the first, at the start of mild contractions, did not differ significantly from that of the last previous analysis at 38 weeks. It is possible that a higher value intervened. The result of the second, made after 24 hours of strong contractions, was the lowest observed in this individual. An increased concentration of the enzyme was found 20 hours later, at the time of delivery, following a very prolonged and arduous labor. In this individual the only postpartum value, 6 days after parturition, had not altered from that at delivery, indicating, perhaps, that the patient had not fully recovered from an unusually difficult labor. In the other 4 cases the postpartum values were lower than any observed prior to the onset of labor. The activity was still low 30 days post partum in the only case studied after involution of the uterus.

No estrogen studies were made on these particular patients but a composite curve of urinary estrogens in 15 normal late pregnancies (13) is included in figure 1. The total active estrogens represent the rate of excretion of estrogens as such. The additional estrogenic activity acquired by zinc hydrogenation (unaccounted for T_{zn} activity) supplies a gauge of the rate of estrogen degradation in the body (19). It is seen that the curves for the diastatic activity of the blood roughly parallel those for the rate of estrogen excretion and are inversely proportional to the rate of estrogen destruction. Our studies have shown that progestin inhibits destruction and facilitates the rate of metabolic conversion of the estrogens (9–15). It would appear, therefore, that the increasing production and utilization of the placental steroids between the 30th and 38th weeks of pregnancy is associated with rising levels of serum diastase; whereas, progestin withdrawal and the increased rate of estrogen destruction which precede and accompany labor are associated with a precipitous drop in diastatic activity, low levels being maintained

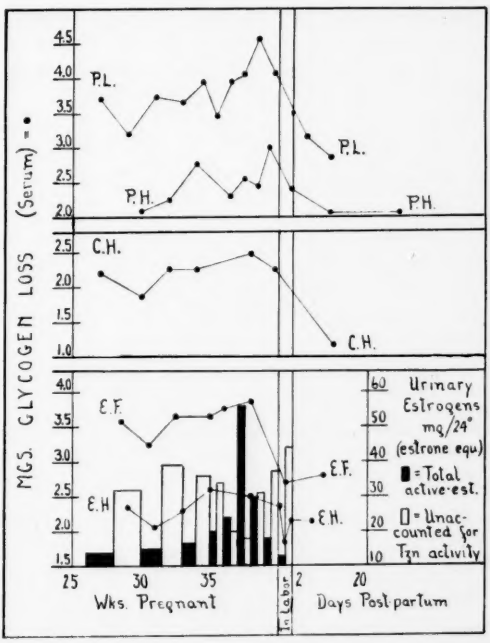

FIG. 1. SERUM DIASTASE IN 5 NORMAL LATE PREGNANCIES AND URINARY ESTROGENS (COMPOSITE CURVES—15 CASES). *P. L.*, aged 20, para I; non-diabetic. *P. H.*, aged 19, para I; non-diabetic. *C. H.*, aged 25, para I; diabetes for 4 years; controlled by 80 to 100 units of protamine Zn insulin daily. *E. F.*, aged 23, para I; diabetes discovered during second half of this pregnancy; controlled by 50 units of protamine Zn insulin daily. *E. H.*, aged 30, para I; diabetes discovered during second half of this pregnancy; controlled by 10 to 12 units of protamine Zn insulin daily.

for as long as one month post partum. In one case of prolonged labor a rise in serum diastase was observed following the initial drop.

The curves for blood diastase in pregnant diabetics (in whom the disease is controlled) do not differ from those of non-diabetic individuals. This same observation holds for the hormone balance (21). There is ample evidence that the diastatic activity of serum is altered in conditions of deranged carbohydrate metabolism such as prevail in cases of un-

controlled diabetes, in depancreatized animals (1, 2, 8, 20) or in normal animals in which hypoglycemia has been induced and maintained by insulin administration (8). The administration of insulin to depancreatized animals, however, restores normal levels of serum diastase. In diabetics, therefore, in whom the diabetes is controlled by insulin administration, as was the case in the 3 patients studied by us, no effect on diastatic activity would be expected. In the summary of our data on serum diastase (table 1) there is no significant difference between serum diastase in the diabetic and non-diabetic pregnancies and the parallel curves in diabetics and non-diabetics indicate that the changes observed in diabetic women during the course of normal late pregnancy, labor and the puerperium, and in late pregnancy complications, were typical of these conditions and in no way influenced by the diabetes itself or by the insulin administered.

The results reported in the literature on serum diastase during normal pregnancy are conflicting. Piano (22), in agreement with our data, finds a gradual rise in diastase up to parturition, with a drop to normal or below during the puerperium. Sugiyama (23, 24) and Goldschmidt-Fürstner (25), on the other hand, report lower values in pregnant than in non-pregnant women, with a rise to normal within 3 to 13 days post partum, while Spitzer (26) and Arneson and Morrin (27) detect no change in the diastatic activity of the blood associated with normal pregnancy or confinement. The latter, however, in agreement with our findings on *E. H.*, did note a rise during labor if the process was long and difficult.

These contradictory results may be accountable in part to the diverse methods employed for serum diastase determination, and in part to the fact that most investigators have based their conclusions on composite findings rather than on successive specimens from the same individuals. Our repeated analyses indicate that although the diastatic activity of the blood for any given individual is fairly constant, there is a wide range over which the values may lie in different individuals. A total of 10 analyses were performed on 6 other women during the last trimester of normal pregnancy. Including these with those presented in figure 1, the normal range of glycogen loss in late pregnancy, from a 20-mg. glycogen solution, using 10 per cent serum dilution, lies between 0.95 and 4.59 mg. (table 1).

TABLE 1. SUMMARY OF DATA ON SERUM DIASTASE IN WOMEN

	No. of Cases	No. of Bloods	Mg. Glycogen Loss[1]	
			Range	Average
Normal late pregnancy				
Before labor	11	44	0.95–4.59	2.85
During labor	3	5	1.85–2.40	2.65
After delivery	5	8	1.18–3.15	2.33
Late pregnancy complications				
Pre-eclampsia & eclampsia	9	26	1.02–4.37	2.21
Fetal death	2	12	1.35–2.36	1.82
Total diabetic pregnancies	7	48	1.02–3.84	2.23
Total non-diabetic pregnancies	15	50	0.95–4.59	2.58
Non-pregnant women throughout cycles	2	29	1.98–4.32	3.28

[1] From a 20-mg. solution of glycogen with a 10% dilution of serum.

Late pregnancy complications. Three patients have been studied before and during the development of complications in late pregnancy. Previous publications from this laboratory have demonstrated that pre-eclamptic toxemia and eclampsia and certain cases of premature delivery or stillbirth are preceded by an abnormal rise in serum chorionic gonadotropin (11, 14). This is accompanied by evidence from urinary analyses of a progressive deficiency of estrogen and progestin culminating in the rapid destruction of estrogen which characterizes *a*) the onset of clinical signs in these diseases (14), *b*) the onset of normal labor (13) and *c*) normal menstruation (10, 15, 19). The hormone findings in all 3 of these patients showed the above abnormalities. Two of them developed pre-eclampsia about 6 weeks after the first rise in serum chorionic gonadotropin. The third, a patient with a history of stillbirth,

was prophylactically treated with estrogen and progestin from the 31st week on, 6 weeks after the first rise in serum chorionic gonadotropin.

The serum diastase curves in these 3 cases are presented in figure 2. In each, the first abnormal increase in chorionic gonadotropin was followed by a steady drop in the diastatic activity of the serum. With two of them, *E. C.* and *F. M.*, this drop came before the 30th week and therefore cannot be considered abnormal when it is compared with the curves for normal pregnancy (fig. 1). With *S. L.*, however, the continued falling off in the level of serum diastase after the 30th week is the exact opposite of the normal findings. At 33 weeks albuminuria, the first clinical sign, was discovered. After 10 days of increasingly severe toxemia with hypertension and subjective symptoms, a transient rise in serum diastase was observed, while a blood taken the next day was again very low in diastatic activity. Labor was induced at 35½ weeks because of severe pre-eclampsia (a blood pressure of 180/110 mm. Hg., a trace of albumin and blurring of vision). A blood specimen taken at the start of mild contractions showed no change in diastatic activity. The baby died 24 hours post partum.

E. C. was the third[2] diabetic patient with toxemia in whom the hormone findings indicated an attempt at self-correction of the estrogen and progestin deficiency. The marked rise of serum diastase after the 30th week coincided with evidence from urine analyses of a corresponding rise in the production and conversion of the sex steroids. Aside from transient edema, this patient had been clinically well up to the 33rd week of pregnancy. At this time, facial edema was apparent and there was slight albuminuria (0.3 gm. per 24 hours). Toxic signs were never more than mild, the highest blood pressure reading being 135/88 mm. Hg. After 10 days of routine hospital care, the edema had disappeared but there was no decrease in albuminuria. At 34½ weeks spontaneous uterine contractions developed but subsided after several hours, at the end of which time a marked drop in serum diastase was observed similar to that found during 3 normal labors (fig. 1). This patient had a history of 2 previous stillbirths. An elective cesarean section was, therefore, performed and the baby, despite severe cyanosis for a few days, survived. A blood taken 3 days after delivery still showed low diastatic activity.

In the case of *F. M.*, a non-diabetic, the blood pressure at the 30th week had risen from 90/60 to 120/80 mm. Hg and there was also a gain of 5 pounds of weight in the course of one

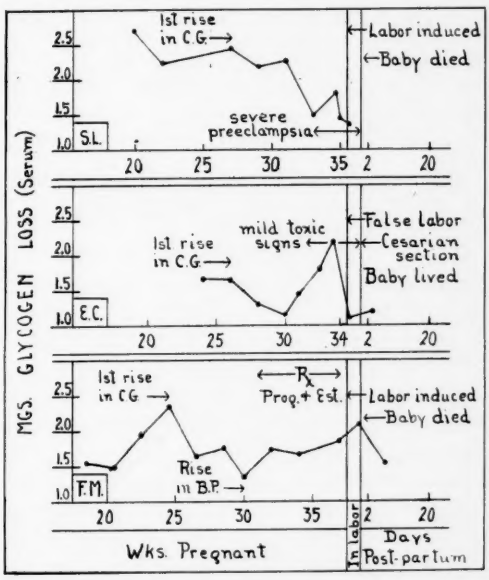

FIG. 2. SERUM DIASTASE IN 3 PATIENTS WITH LATE PREGNANCY COMPLICATIONS. *S. L.*, aged 26, para I; diabetes for 10 years; controlled by 30 units of insulin daily. *E. C.*, aged 38, para 3; diabetes for 19 years; controlled by 64 units of insulin daily. History of arrested tuberculosis and two previous stillbirths. *F. M.*, aged 30, para 2; non-diabetic. History of premature delivery and stillbirth at 28th week of previous pregnancy.

week. Considering the patient's history of stillbirth with identical clinical signs at the 28th week of a previous pregnancy, together with the steadily rising level of serum chorionic gonadotropin and evidence from urine analyses of a marked deficiency of estrogen and progestin, 5 mg. of estradiol and 25 mg. of progesterone were injected daily, starting at the 31st week, in the hope of averting trouble. Injections were continued for 6 weeks, during which time the serum chorionic gonadotropin dropped to normal levels and the urinary findings reflected an approach toward normal metabolism

[2] The findings on one of these have been reported in detail —*A. L.* of figures 1–6 (ref. 14).

of estrogen and progestin. These changes during therapy were accompanied by a steady rise in the diastatic activity of the serum. Injections were discontinued at 37 weeks, and 4 days later labor was induced. A blood specimen taken at delivery showed increased diastatic activity, a finding duplicated at the time of delivery in *E. H.* (fig. 1), one of the normal cases. The baby died 24 hours post partum. Gross examination of the placenta revealed it to be pale,

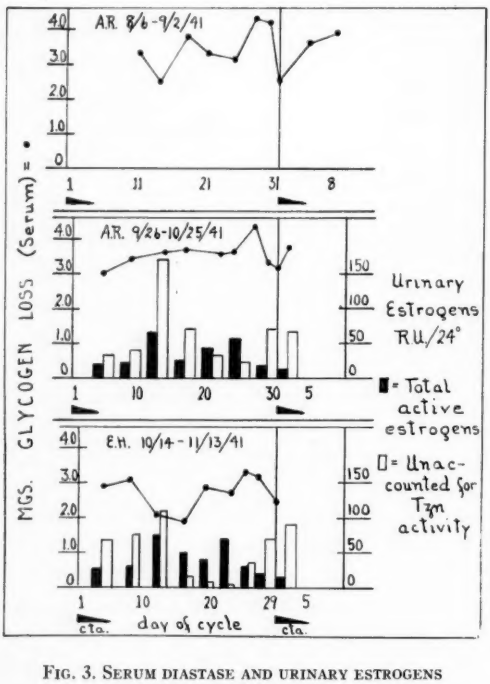

FIG. 3. SERUM DIASTASE AND URINARY ESTROGENS IN MENSTRUAL CYCLES.

firm and thin with many infarcts. Seven days post partum the diastatic activity of the blood had fallen.

Some association between hormone balance and the serum diastase system is definitely indicated by our findings in both normal and abnormal cases. The rise in serum diastase following estrogen and progestin administration to *F. M.* supplies especially convincing evidence in favor of some close relationship between serum diastase and the placental steroids. The data in figure 1 indicate that the peak in diastatic activity in normal pregnancy comes during the last 10 days of gestation, that is, after the peak in sex steroid production and conversion. Similarly, in the 3 abnormal cases, no drop in serum diastase was observed until after the first indication of hormonal imbalance (that is, the abnormal rise in serum chorionic gonadotropin). In other words, the changes in serum diastase appear to follow rather than accompany shifts in hormone balance. In 2 instances when blood was taken at the time of delivery, and in the only case of severe toxemia, a secondary rise in serum diastase was observed.

Aside from the repeated observations on *S. L.*, *E. C.*, and *F. M.*, reported above, a total of 12 analyses for serum diastase were performed upon 8 other women during late pregnancy complications. One had diabetes with premature delivery and stillbirth at 31 weeks. There was no evidence of hormonal imbalance in this individual and 2 blood specimens, one taken a month and the other a week before the accident, revealed no significant change in the level of serum diastase. Of the other 7 patients, all non-diabetic, 2 had severe and 2 mild pre-eclampsia and 3 were eclamptic. No studies were made on these cases prior to the development of toxic signs. The values for serum diastase ranged between 1.37 and 4.37 mg. of glycogen loss from a 20 per cent glycogen solution, using 10 per cent serum dilution. Very high levels were observed in 2 patients from whom blood was taken during convulsions.

As is seen in table 1, the average values in late pregnancy complications are lower than in normal pregnancy before delivery. Considering the wide range over which the values may be distributed, the significance of this difference is questionable. Arneson and Morrin (27) found serum diastase values distinctly below normal in 35 per cent of their determinations on patients with pre-eclampsia, and Gray and his collaborators (2) also found subnormal values in toxemia of pregnancy.

Menstrual cycles. Two women have been studied throughout 3 complete menstrual cycles. Both ovulated regularly, as indicated by their menstrual histories and by repeated studies on the cyclic excretion of estrogens, some of which have been previously reported (15). *A. R.*, aged 28, unmarried, is a case of essential dysmenorrhea and *E. H.*, aged 41, married, is still having normal periods at 27 to 29-day intervals. Urinary estrogens were measured in 2 of the 3 cycles during which repeated bloods were obtained for diastase determinations. In figure 3 the results are tabulated, total active estrogens (that is, estradiol plus estrone plus estriol) being recorded and also

'unaccounted for' T_{zn} activity, an index of the rate of estrogen destruction in the body.

In each of the 3 cycles a premenstrual increase in diastatic activity followed the luteal phase, that is, the period of increased progestin secretion resulting in maximum conversion and minimum destruction of the estrogens. At the start of menstruation, following estrogen and progestin withdrawal with the consequent marked increase in the rate of estrogen degradation, a drop in serum diastase was noted. In 2 cycles a drop in serum diastase was also observed at the probable time of ovulation, 14 to 18 days before the start of flow. At this time a peak in the excretion of active estrogens may be noted, but, unlike the second peak during the luteal phase, this early rise is accompanied by evidence of a striking increase in the rate of estrogen degradation, due probably to increased estrogen secretion in the absence of adequate luteal control. It is possible that an intermenstrual drop in serum diastase was missed during the second cycle on *A. R.* Each urine specimen from this individual represented a 3-day period of excretion and would, therefore, include transient marked changes that might have preceded or followed the moment when the blood was taken.

The luteal phase of the menstrual cycle is analogous to the period in late pregnancy (around the 38th week) when the greatest production and most efficient metabolism of the female sex steroids appears to prevail (13). In both pregnant and non-pregnant women this type of metabolism is followed by a rise in serum diastase. The increased rate of estrogen degradation which characterizes the start of menstruation (10, 15, 19) is also a consistent finding before and during labor (13) and in the late pregnancy toxemias (14), and in each of these situations a decreased diastatic activity of the serum has been observed. In the tabulated results at the probable time of ovulation in *E. H.* it is particularly indicated that any correlation between the estrogens and serum diastase is more closely related to the rate of estrogen degradation than to the level of secretion or excretion of active estrogens.

The average value for glycogen loss from the sera of these 2 women is higher than those from pregnant individuals (table 1). We question the significance of this observation, although it is in agreement with those reported by Sugiyama (23, 24) and Goldschmidt-Fürstner (25). Only by averaging a very large number of single observations or by following changes in the serum diastase in a single individual throughout a menstrual cycle, a pregnancy and a confinement could the actual levels during these conditions be compared.

DISCUSSION

Considering the number of factors which may influence the diastatic activity of the blood, any interpretation of our data must be purely speculative.

Certain effects of the estrogens upon the secretory activity of the anterior hypophysis have been well established, and our own studies of estrogen metabolism in women have led to the hypothesis that estrogen degradation is an important factor in the growth and maturation of ovarian follicles (13, 15), presumably through pituitary stimulation. Cope, et al., have shown that hypophysectomy causes a sustained increase (6) and the injection of anterior pituitary extract a decrease (8) in the diastatic activity of the blood of dogs. The drop in serum diastase in women, therefore, at the times of increased estrogen destruction, that is, at ovulation, menstruation, before and during labor and in cases of late pregnancy toxemia, might be interpreted as reflecting pituitary stimulation. The increased gonadotropic potency of the urine observed at these times (14, 21, 28, 29) is in keeping with this interpretation, although in pregnancy the gonadotropin is of chorionic rather than pituitary origin. Conversely, the rise in serum diastase following periods of maximum conversion and minimum destruction of the estrogens, that is, after the luteal peak of the cycle and during the last 10 days of pregnancy, might be considered as reflecting decreased gonadotropin secretion due to withdrawal of the stimulus of estrogen degradation.

Adrenalectomy and the administration of adrenal cortical extracts are reported (7) to have even more striking effects upon the diastase system of dogs, the former causing a sustained rise and the latter a fall in the level of this enzyme in the blood. We have previously postulated that the local vascular changes of the menstruating endometrium, as well as the generalized vascular pathology of pre-

eclampsia and eclampsia, may be due to the liberation of some 'toxin' consequent upon an increase in estrogen degradation (14). There is considerable evidence that increased adrenal cortical activity is a normal response to any damaging influence. The drop in serum diastase at the time of ovulation, at the start of flow, in late pregnancy toxemia and just before and during labor would thus be in keeping with the idea that increased steroid degradation supplies some 'damaging influence' which stimulates the so-called (30) adrenal 'alarm reaction.' And the secondary rise in serum diastase observed at the end of labor, particularly when the process is long and difficult, and in the acute stage of severe pre-eclampsia and eclampsia, might be considered as reflecting a degree of cortical exhaustion. We have previously suggested that adrenal exhaustion might contribute to the alarming rapidity with which pre-eclampsia and eclampsia may develop (31).

Direct substitution for cortical secretion by progesterone is another possibility that must be considered in the interpretation of our data. Progesterone in sufficient dosage will prolong the life of adrenalectomized animals (32) and progesterone administration to rats has been shown to cause atrophy of the adrenal cortex (33). Raised titers of serum diastase, therefore, at the times of increased progesterone secretion, as, for example, during the luteal phase of normal cycles and between the 30th and 38th weeks of normal pregnancy, might be accountable to adrenal quiescence as a direct result of progesterone inhibition of cortical secretion. Conversely, the drop in serum diastase at menstruation and labor and in late pregnancy toxemia and the sustained low levels after delivery might reflect increased activity of the adrenal cortex called forth by progesterone withdrawal.

The above considerations are presented merely as possible endocrine factors involved in the observed changes in the level of serum diastase in women. Cope, et al., have noted a species difference between the dog and rabbit in the activity of the serum diastase system (7, 8), and have been unable to correlate any chronic pituitary or adrenal abnormalities in human beings with consistent changes in the level of this enzyme in the blood (17). Furthermore, there is evidence (8) that variations in thyroid activity and in the internal secretion of the pancreas alter the level of serum diastase. These glands may well be influenced by changes in the ovarian and placental hormones inasmuch as they are interrelated with pituitary activity.

Factors other than the endocrines must, of course, be considered in the interpretation of our data. Low diastatic activity of the blood in diseases involving liver damage has been reported (1–5). Heilig and Kantienger (34) have found evidence of impaired liver function at the time of menstruation in normal women, and liver damage is often, if not consistently, a feature of toxemia of pregnancy. There is no indication that liver changes precede the onset of menstruation or the clinical manifestations of late pregnancy toxemia, as do the changes in sex steroid metabolism and the drop in serum diastase, although this possibility cannot be ignored. Gray, et al. (2), have ascribed their finding of subnormal values for serum diastase in pre-eclampsia entirely to impaired liver function.

Finally, there is the well-known influence of the external secretion of the pancreas upon the level of serum diastase to consider. No direct relationship between this and the glands of internal secretion has been established. An increased diastatic activity of the blood has been reported, however, following the injection of acetylcholine into dogs (35), the response being inhibited by previous treatment with atropine. This effect has been ascribed (1, 20) to spastic closure of the pancreatic ducts due to the action of acetylcholine on the parasympathetic nervous system. Reynolds (36) has demonstrated a transient increase in the acetylcholine content of rabbits' uteri following estrogen administration and presents good evidence that the initial hyperemia and vasodilatation of the uterine reaction to estrogen is the result of the acetylcholine-liberating property of this hormone. Our data in women show in most instances a parallelism between the level of estrogen excretion and the level of serum diastase. There are, however, notable exceptions to this generalization. At the time of the first peak in estrogen excretion in the normal menstrual cycle, for example, a drop in serum diastase

was observed. The investigations of Zeller and Birkhaüser (37, 38, 39) on the relationship between cholinesterase and the sex hormones suggest that this enzyme, which characteristically destroys acetylcholine, may be increased in those situations in which an increase in the rate of estrogen degradation would prevail. At the time of ovulation, therefore, despite the evidence for an increase in the level of circulating estrogen, liberation of acetylcholine may be overshadowed by an increase in cholinesterase accountable to a temporary increase in the rate of estrogen destruction. All of our data accumulated during the menstrual cycle and pregnancy could thus be interpreted as reflecting an interplay between estrogen conversion and degradation and the production and destruction of acetylcholine with resultant changes in the externally secreting cells of the pancreas. This possible relationship between the course of estrogen metabolism and acetylcholine production and destruction is of particular interest in connection with the local vascular changes of the endometrium during the ovarian cycle (40), the variations in vascular supply to the placenta prior to the onset of labor (41) and the generalized vasomotor disturbances which characterize late pregnancy toxemia.

The composite findings presented in table 1 demonstrate the wide range over which the levels of serum diastase may vary. Only by repeated analyses would changes in activity, under the conditions studied, have been revealed. Furthermore, the alterations in diastase which characterize pancreatic disease appear to be related to acute episodes, normal levels being re-established as the condition becomes chronic (42). Cope, et al. (8), have evidence for a 'compensatory mechanism' in the serum diastase system of dogs, and in the case of our patient, *S. L.*, a severe pre-eclamptic, the rise when the patient became alarmingly ill was transient in nature. These considerations emphasize the importance of repeated analyses as against single observations in the study of diastatic activity.

SUMMARY AND CONCLUSIONS

Diastatic activity was measured in 98 specimens of blood from 22 women during the last 10 weeks of pregnancy, labor and the puerperium. There were 11 normal pregnancies, 6 cases of pre-eclampsia, 3 eclamptics and 2 cases of fetal death. The findings are compared with changes in estrogen metabolism during these episodes.

Twenty-nine analyses were performed upon blood from 2 women throughout 3 complete menstrual cycles, during 2 of which urinary estrogens were determined.

Repeated analyses indicated a rise in serum diastase after the 30th week of normal pregnancy to a peak within 10 days of delivery, a drop just before and during labor, and low values for as long as 1 month post partum. A progressive decrease preceded and accompanied those late pregnancy complications associated with imbalance of the placental hormones, this effect being counteracted by estrogen and progesterone administration and by self-correction of the steroid deficiency.

In women with normal menstrual cycles a rise in serum diastase during the luteal phase reached a peak a few days before the onset of flow. Menstruation was preceded and accompanied by a precipitous drop. In two instances there was a drop at the probable time of ovulation.

In both pregnant and non-pregnant women, the decreased rate of estrogen degradation which characterizes increased progestin secretion was followed by a rise in serum diastase; whereas a drop in enzymic activity was associated with evidence from urine analyses, of increased estrogen degradation due to progestin withdrawal or deficiency.

Possible endocrine interrelationships to account for these findings are discussed.

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Treatment of Menstrual Disturbances in Adolescent Girls

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MENSTRUAL disturbances in adolescence consist of two varieties, those of rhythm and those of amount and duration of bleeding. Both types of disturbance usually are temporary since ultimately the girl's own ovaries mature sufficiently to establish normal menstrual rhythm and flow. While menstrual irregularity presents no clinical problem, excessive or persistent bleeding does require medical intervention. Two distinct therapeutic approaches have been successfully employed in such cases, but each has serious disadvantages. When the bleeding is of serious proportions, surgical treatment, i.e., curettage and packing of the uterus, and even laparotomy for excision of ovarian cysts has been undertaken. It is obviously undesirable to submit patients to the risk of anesthesia and operation upon the genital tract if some way of avoiding it is available.[2] For less serious bleeding, male sex hormone in large doses has been successfully used; this therapy involves the risk of inducing such masculinization as acne, hirsutism, hoarseness of voice and enlargement of the clitoris.

The disadvantages of the methods for treating menometrorrhagia noted above, coupled with the scattered reports of successful therapy of this condition with progesterone and estrogen, led us to investigate systematically a series of patients complaining of severe, irregular bleeding. It was hoped that with the vaginal smear technic (1, 2) as a guide, we could infer whether the bleeding occurred from an atrophic or from a hyperplastic endometrium. The rationale of therapy was to be the induction of *a*), endometrial proliferation with estrogens, if the bleeding was from an atrophic endometrium or *b*), a transformation from a hyperplastic to a secretory endometrium with progesterone, if the bleeding was from a presumably hyperplastic endometrium.

The vaginal smear which indicates the presence of a hyperplastic endometrium consists of plaques of cornified epithelial cells (*type IV*); the vaginal mucosa, in response to high and persistent levels of estrin, has grown so thick that its superficial cells (from which the vaginal smears are taken) are partially keratinized and degenerated (fig. 1). In contrast the vaginal smear which accompanies an atrophic endometrium consists of basal epithelial cells (*type VIII*) which are small and round with large vesicular polychromatic nuclei (fig. 2). Either through refractoriness of the end organ, or because there is an absolute lack of estrogen, the vaginal mucosa has remained thin so that cells from its superficial layers are the actively dividing and growing cells; i.e., basal cells. Each of these smear types is so distinct from that occurring normally during menstruation, that no difficulty should be encountered in making the differentiation. The normal vaginal smear at the time of menstruation contains blood, cells and cell fragments (*types VII, I, II*) from the vaginal epithelium which is neither atrophic nor hypertrophic; that is, the cell nuclei show only early evidences of pyknosis while the cytoplasm is somewhat granular and frequently folded. By keeping in mind these three distinct vaginal smear types it is relatively easy to determine whether any particular episode of bleeding is normal or pathologic, and if the latter, whether it occurs from an atrophic or hyperplastic endometrium.

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[2] This report does not imply that menometrorrhagia due to ovarian tumors can be treated successfully by non-surgical means. Vaginal smears may be used to diagnose cases which do not require surgery.

In those of our cases in whom a highly cornified vaginal smear was found (indicating a hyperplastic endometrium), progesterone was used to convert the hyperplastic endometrium to the secretory type and thus stop the bleeding. It was to be expected that upon interruption of progesterone therapy a normal and self-limited menstruation would occur. It is important to remember this, for progesterone-withdrawal bleeding should not be mistaken for a recurrence of metrorrhagia. An example of this type of case follows:

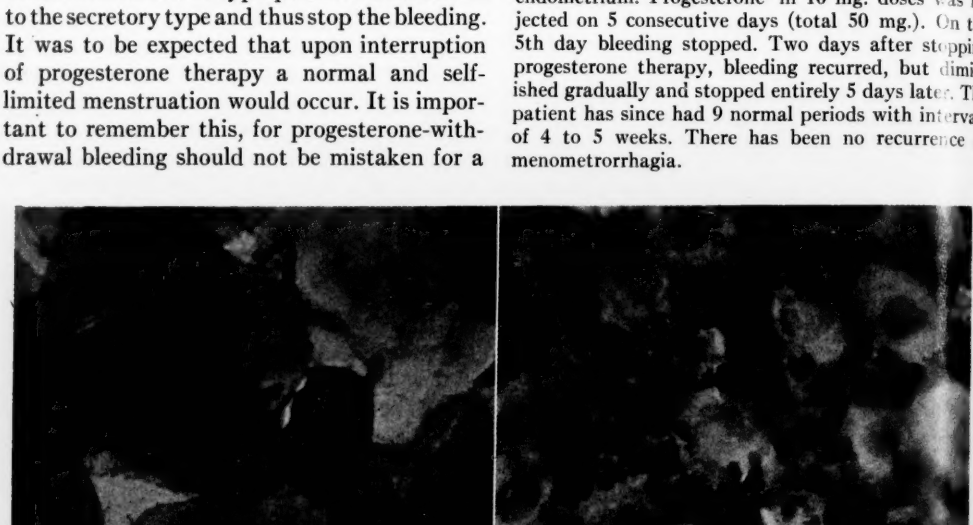

FIG. 1, *left*. Vaginal smear consisting of plaques of cornified epithelial cells (*type IV*).
FIG. 2, *right*. Vaginal smear consisting of basal epithelial cells (*type VIII*), which are small round cells with large vesicular polychromatic nuclei.

CASE REPORTS

The patient, a 14-year-old girl, menarche at 12 years, was first seen in the Clinic because of profuse bleeding of two weeks' duration. Vaginal smears consisted of plaques of cornified cells (*type IV*) resembling the smear shown in figure 1, plus an abundance of red blood cells. She was given pregneninolone[3] (oral progesterone) 10 mg. per day for 14 days. The bleeding subsided, but did not stop. When therapy was interrupted bleeding again became more profuse, threatening to exsanguinate the patient. The vaginal smear now seemed more nearly normal for menstruation, consisting of cell types *VII, IV, III*. The flow continued, however. A week later the smear was again that of hyperestrinism (*types IV, III*) and suggested a recurrence of menometrorrhagia from a hyperplastic endometrium. Progesterone[3] in 10 mg. doses was injected on 5 consecutive days (total 50 mg.). On the 5th day bleeding stopped. Two days after stopping progesterone therapy, bleeding recurred, but diminished gradually and stopped entirely 5 days later. The patient has since had 9 normal periods with intervals of 4 to 5 weeks. There has been no recurrence of menometrorrhagia.

In this patient and in three others with similar histories and findings it has been possible to interrupt the episodes of severe bleeding by means of adequate progesterone dosage, as illustrated by the following case histories.

Case 2, a negro girl, aged 12, was seen in her third menstrual period, 9 months after menarche. The period was excessively profuse and was continuing beyond 8 days. The developmental history was normal. No abnormalities of body size or shape were noted. Rectal examination revealed a uterus of normal size, with a firm, erect corpus. The adnexae were not palpable. The Hb was 60 per cent; erythrocytes, 3.8 million; leucocytes, 8.6 thousand with a normal differential count. Vaginal-smear cell types *IV, III* and *VII* were found. Testosterone propionate in 25 mg. doses was given and repeated at 2-day intervals until

[3] Pregneninolone (Lutocylin) and progesterone (Lutocylol) were supplied by the Ciba Pharmaceutical Products, Inc., Summit, N. J.

100 mg. had been injected. Bleeding stopped in one week. The vaginal-smear cell types were *VII, III, II, I.* She returned 33 days later with a recurrence of profuse bleeding. Progesterone, 10 mg. per day for 5 consecutive days was given and the bleeding stopped. A single 'gush' of blood occurred the day after cessation of progesterone therapy. Spotting continued 4 days longer. She has had 4 normal menstrual periods since the last treatment.

Case 3, a white girl, aged 11 years, was seen on the 22nd day of profuse menstrual bleeding which required the use of 12 sanitary napkins per 24 hours. Menarche had been at 9½ years of age. The menses had been irregular in interval and duration since their onset. The girl appeared older than her stated age, with large breasts and adult external genitalia. She was somewhat obese, but pale and weak. The Hb was 50 per cent, erythrocytes 3.3 million. Rectal examination disclosed a large, boggy uterus characteristic of that in the premenstrual phase of a normal adult woman. Vaginal smear showed cell types *IV, VII.* Progesterone, 5 mg. b.i.d. was given until bleeding stopped, requiring a total dose of 30 mg. There were some mild cramps and spotting for 2 days following interruption of therapy, then 5 days of 'normal' menstruation requiring the use of 6 to 8 pads per day. Bleeding stopped spontaneously on the 8th day. The next menstrual flow began 23 days later, and was so profuse as to frighten the patient and her mother. She was again given progesterone intramuscularly, 5 mg. b.i.d. until a total dose of 25 mg. was given. The bleeding diminished during the therapy; spotting continued for 3 days after interrupting the injections. The patient has since had 3 normal menstrual periods.

Case 4, a negro girl 12 years old, whose menarche had occurred a year before, was seen during her second episode of menorrhagia. She had had curettage (at which time a hyperplastic endometrium was found) 4 months before. The vaginal smear was *type IV, VII.* She was given anhydroxy-progesterone tablets, 10 mg. *t.i.d.* for 7 days. At that time the vaginal smear was *type VII, IV, V* and the bleeding had diminished. An endometrial biopsy showed 'presecretory' endometrium. The medication was continued for 3 days longer, and the bleeding stopped entirely. A moderate flow recurred 6 days later and continued, in diminishing amounts, for 5 days. The vaginal smear was *type VII, III, II, V.* No further bleeding occurred for 6 weeks; then a normal period occurred. Thirty-two days later menorrhagia began again. It was interrupted by injections of progesterone, 10 mg. per day for 5 days. Moderate withdrawal-bleeding followed interruption of therapy. There has been no recurrence of menorrhagia in 3 months since the last therapy.

It is not claimed that progesterone will effect a cure, but it may be used to tide the girl over a period until the girl's own ovaries begin their cyclical secretion of progesterone. In our experience, 50 mg. of progesterone distributed over a 5-day interval, has been the only consistently effective therapy. The effectiveness of oral progesterone in doses as high as 30 mg. per day for 10 days has not equalled that of 50 mg. of injected progesterone.

Bleeding due to hypoestrinism occurs occasionally. The bleeding starts at the time of a normal menstruation but fails to stop. Whereas, in the normal girl, bleeding stops upon reconstitution of the endometrium under the influence of estrogen, in these patients there is a delay in reconstitution of the endometrium. The stimulus to growth (estrogen) is either inadequate or lacking. The bleeding, therefore, continues indefinitely until some stimulus to healing is provided. In such patients the vaginal smear offers characteristic criteria. It resembles that of the normal prepubertal girl. The epithelial cells are from the basal layer, small, round cells with large vesicular nuclei. When such a smear is encountered in a persistently bleeding patient past the menarche, the logical therapy would seem to be to stimulate the proliferative and reparative processes in the endometrium by giving estrogen. The following case history provides a typical example.

A 15-year-old girl, menarche at 12 years, was brought in by her mother, who reported that bleeding and spotting had occurred continuously and persistently for the past 7 months. During all of this time the girl had had to wear sanitary napkins and had been advised to refrain from sports and exercises. She was moderately anemic. Her growth and appetite were normal. The persistent bleeding was not only a source of worry, but was a drain upon the hemoglobin and iron. Rectal examination revealed no pelvic abnormalities. The external genitalia were normal. The hymen was intact. The vaginal smear consisted of blood, leucocytes, and basal (*type VIII*) cells. She was given 0.1 mg. of stilbestrol[4] orally each day. In 3 weeks the bleeding stopped. Stilbestrol therapy was continued for 6 weeks longer and then was gradually tapered off, one tablet being given on alternate days and then every third day. Normal menstrual periods occurred during the course of treatment. In the following 6 months, periods have recurred at regular intervals and the patient has been free from menorrhagia.

Case 6 was a 12-year-old girl at menarche. This, her first menstrual period, had begun 3 weeks previously as a moderate flow, but spotting had persisted since. Developmental history and physical examination revealed no abnormalities. The Hb was 75 per cent. The

[4] The stilbestrol was supplied through the courtesy of Dr. E. Gifford Upjohn, The Upjohn Co., Kalamazoo, Mich.

blood count showed 4.2 million erythrocytes, 8.2 thousand leucocytes with a normal differential count. The vaginal smear showed cell types *VII, VIII, I*. She was at once given an injection of 1 mg. of estradiol dipropionate[5] and 0.12 mg. of stilbestrol[6] was prescribed to be taken each night before retiring. Bleeding stopped in 10 days. The vaginal smear types were *VII, II, I*. She continued the same medication until 4 mg. had been taken. Three weeks later a normal period began; 4 mg. of stilbestrol in 0.12 mg. doses in liquid form[6] was again prescribed. A 'brownish' vaginal discharge persisted for 10 days after the 4-day normal menstruation. She has had no further therapy, and there has been no recurrence of menorrhagia in 9 subsequent menstrual periods.

Case 7 was a 13-year-old girl, whose menarche had occurred at 11 years. The history revealed Pott's disease which had healed without deformity after appropriate treatment. This was the 5th day of the 3rd episode of menorrhagia, which in this patient began as a 'normal' period with profuse flow on the second and third days, tapering off to a flow requiring about 4 napkins per day for 10 to 12 days more. Vaginal smear cell types were *VII, I, VIII, V*. She was given stilbestrol, 0.5 mg. per day. In one week the vaginal smear showed cell types *III, VII, II, IV*. The dosage of stilbestrol was reduced to 0.1 mg. per day, and therapy continued. Bleeding stopped on the 10th day; this may have been a coincidence. Stilbestrol was continued at 0.1 mg. per day for a total of 100 days. She had 2 normal menses during the period of therapy. Therapy was then interrupted. She has continued to have normal menstrual periods every 4 to 5 weeks for nearly a year.

DISCUSSION

While, in general, control of menometrorrhagia, regardless of its etiology is possible by surgery or by giving large doses of male hormone, these types of therapy are undesirable for the reasons previously outlined. The problem was therefore investigated from the point of view of possible treatment with one or another of the female sex hormones. This proved possible since the vaginal smear permitted determination of the cause of the bleeding. Classical methods such as endometrial biopsy, curettage, or hormone assays are laborious and expensive, and may involve invasion of the genital tract, while the vaginal smear technique is rapid, easy, and acceptable to the patient. The difference between the cell types in normal menstrual smears and in the smears obtained, respectively, from cases of hyperestrinism and hypoestrinism are so characteristic as to leave no doubt concerning the diagnosis.

Thus, in three patients (ages 12, 13, 15) whose vaginal smears showed the presence of basal epithelial cells, indicating an atrophic endometrium, the bleeding was controlled by estrogenic therapy. And in the four patients (ages 11, 12, 12, 14) whose vaginal smears were predominantly plaques of cornified epithelial cells with pyknotic nuclei, progesterone therapy controlled the bleeding. It should be emphasized that upon withdrawal of progesterone therapy bleeding occurs. This bleeding, in contradistinction to the bleeding for which the therapy is given, is self-limited and resembles that of a normal menstrual period. The use of progesterone should not be discarded simply because bleeding recurs upon withdrawal; such bleeding is expected.

While cases of menometrorrhagia are not very frequent in pediatric practice, when they do occur they present serious problems. The diagnostic aid obtained by use of the vaginal smear technique allows definitive diagnosis and treatment on the basis of etiology. The treatment may be continued until such time as the patient's own ovaries mature sufficiently to prevent the recurrence of menometrorrhagia.

SUMMARY

Seven patients, aged 11 to 15 years, presenting the complaint of severe and/or prolonged menstrual bleeding were studied by the vaginal smear technique. On the basis of the cell types in the smears, it was possible to separate the cases into two groups: the one to be treated with estrogen, the other with progesterone.

The bleeding in all patients was successfully controlled by the appropriate therapy.

I am glad to acknowledge the advice and criticism of Dr. S. Soskin, Director of the Department of Metabolic and Endocrine Research.

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[5] Estradiol dipropionate (Di-ovocylin) supplied through the courtesy of the Ciba Pharmaceutical Products, Inc., Summit, N. J.

[6] Stilbestrol (Elixir Stilbestrol) 1 mg. per fluid ounce was supplied through the courtesy of Dr. E. Gifford Upjohn, The Upjohn Co., Kalamazoo, Mich.

A Case of Secondary Amenorrhea

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THE RESULTS of treatment of secondary amenorrhea are often a source of dissatisfaction both to the patient and her physician. We are reporting a case of secondary amenorrhea in which a careful diagnostic survey was completed and an unusually long and tedious course of therapy was given before a completely successful result was achieved.

CASE REPORT

The patient was first seen in March, 1941, at the age of 17. She was a thin, pale, white schoolgirl who had not had a menstrual period since June, 1940. Menarche occurred at 13 years of age. She had her first 3 periods at 6-month intervals and then had regular 6-week menstrual cycles which terminated abruptly in June, 1940. Her periods had been normal in duration and were never painful. She had never been operated upon and had had only the usual childhood diseases.

Physical examination. Physical examination revealed a thin, white girl with no endocrine stigmata. The weight was 103 lb.; height, 62 inches. The anthropometric measurements were normal. The breasts, external genitalia and hair development were of normal appearance. She was mentally alert but phlegmatic. With the exception of a deviated septum and nasal polyps, the examination revealed no abnormalities. A vaginal examination was not made because of an unruptured hymen, but a rectal examination showed that the pelvis was normal except for an unusually small, hard uterus which was in the normal anteflexed and anteverted position.

Laboratory data. Temperature, pulse and respiration were normal. The blood pressure was 104 mm. Hg systolic, 62 diastolic. Blood and urine examinations gave values within the range of normal. The basal metabolic rate was minue 1. A vaginal smear indicated a *Grade III* reaction of the vaginal mucosa. The Wassermann reaction was negative. The sugar tolerance was high; a flat curve was obtained after the ingestion of 100 gm. of dextrose. Roentgenograms of the sella turcica revealed no abnormalities. The visual fields were entirely normal. Two gonadotropic hormone investigations of 48-hour urine collections were made by the tannic acid precipitation procedure of Levin and Tyndale and assayed using the immature mouse uterine weight method. The urine specimens were collected one week apart and both yielded less than 10 mouse uterus units per 24-hour collection. Three assays for daily urinary estrogens were made by Kurzrok's method. They showed an average of 5 rat units per liter.

Treatment. The patient was first given a series of injections of estrogen in an attempt to increase the size and responsiveness of the womb. Ten injections, each 0.33 mg. of estradiol benzoate[1] were given in 3 weeks. These were followed immediately by daily injections for 5 days of 10 mg. of progesterone.[1] The patient bled for 4 days, the flow beginning 54 hours after the last injection of progesterone. Two weeks after the last day of vaginal bleeding, she was given a second series of 5 progesterone injections without preliminary estrogenic medication. Sixty-five hours after the final injection, a flow began which lasted for 3 days. A third and identical series of progesterone medication was given two weeks later without a preceding course of the estrogens. This did not produce vaginal bleeding.

The patient was permitted to go without medication for 6 months during which time she did not menstruate again. In November, 1941, she was given a course of 'stimulative' roentgen-ray exposures to the pituitary gland and ovaries. She received 600 r to the right temporal region and 400 r to the left temporal region in 5 exposures. The ovaries received 8 irradiations during one month: 300 r to the right anterior pelvis and 300 r to the left anterior pelvis. The pituitary irradiation factors were 200KV; 4 ma; ½ Cu plus 1 Al filter. The pelvic factors were 200 KV; 4 ma; 1 Cu plus 1 Al filter. All treatments were given at 50 cm. These treatments did not result in any further bleedings, although she suffered a temporary alopecia about her temples.

Two months after the last roentgen exposure, the patient was started on 3 courses of combined oral estrogen and progesterone medication. Each course began with daily doses of 1.0 mg. of stilbestrol for 10

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[1] The estradiol benzoate (Dimenformon Benzoate), progesterone (Progestin) and the pregneninolone (Progestoral) were supplied by Dr. Leo Pirk of Roche-Organon Inc., Nutley, N. J.

days and was immediately followed by daily doses of 70 mg. of pregneninolone[1] for 5 days. The interval between the courses of therapy was approximately 3 weeks. Each series of oral medication resulted in an adequate vaginal bleeding. The last flow occurred on March 25, 1942.

The patient menstruated spontaneously in April, 1942, and has menstruated at 6-week intervals, without medication, up to the present time (October, 1942). The menstrual periods are of satisfactory duration and intensity. It is interesting that she has reverted to the 6-week intermenstrual interval exhibited before amenorrhea had become established. She is still at school, does approximately the same amount of work and has not had any unusual economic changes.

SUMMARY

Two uterine bleedings were induced in a young schoolgirl with secondary amenorrhea of 8 months' duration by the parenteral use of progesterone. A spontaneous, normal, menstrual rhythm was not produced. A series of so-called 'stimulation' roentgen exposures to the pituitary and the ovaries was unsuccessful in establishing menstruation. A third series of therapeutic attempts using the combination of stilbestrol and pregneninolone orally was followed by menstruation recurring spontaneously at 6-week intervals.

Vaginal Smear Technic

[A Review Article]

Its Use in the Diagnosis of Ovarian Failure, as an Index to Efficacy of Endocrine Therapy and as a Human Assay Method

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THE VALUE of the vaginal smear method for the study of ovarian functions is based upon the response of the vaginal epithelium to the hormones of the ovary. This relationship has been long evident from comparison of the structure of the undeveloped vagina in childhood and the atrophic mucosa of senility with the histologically normal, fully developed epithelial structure characteristic in sexual maturity; it has more recently also been indicated by the apparent occurrence of rhythmic alterations of the vaginal epithelium during the menstrual cycle. These changes have been accepted by many as proof of a human vaginal cycle. Papanicolaou's publication in 1933, supporting this belief, indicated that such cyclic variations could readily be demonstrated in stained smears of human vaginal fluid (1). A later publication, with Shorr in 1936, described the vaginal cytology of the menopause and advocated the vaginal smear method for the diagnosis of ovarian failure, at the same time demonstrating clearly the morphologic transformation of the vaginal fluid following adequate estrogen therapy (2).

The potential importance of this simple means for investigating ovarian functions and failures, and its evident practical value in gauging therapeutic responses was at once apparent. The following critical review is presented in an effort to appraise the usefulness of the vaginal smear method as judged from pertinent publications in the available literature.

VAGINAL SMEAR TECHNICS

Since the original method for obtaining and staining vaginal smears was described by Papanicolaou, several modifications of technic have been proposed to simplify this procedure. The technical details and the microscopic features of the various staining methods are summarized briefly.

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Ehrlich's Hematoxylin-Eosin-Water Blue Stain (Papanicolaou, 1)

Technic. Secretion obtained from the vagina by means of a curved glass pipette is spread upon glass slides which are immediately immersed in a jar containing equal parts of 95 per cent alcohol and ether. After fixation (½ to 1 hour) the smears are stained with hematoxylin, eosin and water blue in the following manner:

Slides are first stained with Ehrlich's hematoxylin for about 5 minutes, then carried into running water for at least a quarter of an hour. After this, they are stained with a 0.5 per cent eosin for about 3 to 4 minutes, rinsed well in water, and finally stained for 1 minute in a 0.5 per cent solution of water blue. Following this they are rinsed well in water and then carried through 50 per cent, 70 per cent, 80 per cent, 95 per cent and absolute alcohol into xylol and mounted in Canada balsam. The stain is permanent and does not fade easily.

Microscopic appearance. The author describes the preparation as follows.

This secures a sharp outline of the various cell types with a variety of shades from an intense blue to an eosin-red. The cornified cells, which are almost constantly present in the vagina, take an intense eosin color and are sharply differentiated. On the other hand, cells derived from the deeper vaginal layers, which are free from cornification, are stained strongly blue. Partly cornified cells show various shades of purple. Superficial secretory cells, containing mucus, display a typically characteristic bluish-purplish tone. Leucocytes, as well as bacteria, are also sharply outlined. Eosinophiles show distinctly red granulation. Mucus takes a bluish or purplish shade. The contrasts are sharper than with ordinary hematoxylin-eosin and the cornified cells are particularly differentiated. This is important for the vagina, in which the cornification is very pronounced, especially during certain periods of the cycle.

Modified Masson Trichrome Stain (Shorr, 3)

Technic. The vaginal secretion, aspirated by means of a dry pipette with rubber bulb attached, is expelled

onto a glass slide. While still wet, the slide is fixed in equal parts of ether and 95 per cent alcohol. Fixation for 1 or 2 minutes is adequate. Instead of imported stains (Ponceau de Xylidene and Light Green) Shorr later substituted domestic Biebrich Scarlet and Fast Green FCF for staining.

(1) From fixing solution, carry through alcohols to water; stain with Harris' hematoxylin for 2 minutes, and wash in running water for 5 minutes. (2) Instead of the Ponceau de Xylidene-Acid Fuchsin-Orange G solution, 1 per cent Beibrich Scarlet, water soluble (Nat'l Aniline and Chem. Co.) and 0.4 per cent Orange G in 1 per cent acetic acid. Stain 1 minute and rinse in water. (3) In place of the 3 per cent phosphotungstic acid mordant, a mixture of equal parts of 5 per cent phosphomolybdic and phosphotungstic acids. Mordant 1 minute and rinse. (4) In place of 0.3 per cent Light Green, a 0.25 per cent solution of Fast Green FCF (Nat'l Aniline and Chem. Co.) in 0.3 per cent acetic acid. Stain 2 minutes. Do not rinse. (5) Differentiate in 1 per cent acetic acid for 1 minute, carry through alcohols to xylol and mount in damar.

A single differential stain incorporating all the components of the trichrome method described above, with the exception of hematoxylin, was recently recommended by Shorr for clinical investigations (4). The greatly simplified technic is as follows.

1. Aspirate the vaginal secretion by means of a dry pipette with rubber bulb attached, and expel onto a glass slide. 2. Fix, while wet, in equal parts of ether and 95 per cent alcohol. Fixation for 1 or 2 minutes is adequate. 3. Stain for approximately 1 minute in Solution S3. 4. Carry through 70 per cent, 95 per cent and absolute alcohol, dipping slide 10 times in each solution. 5. Clear in xylol and mount in damar.

The composition of the single differential stain (S3) is as follows:

ethyl alcohol (50 per cent)	100.0	cc.
Biebrich Scarlet (water sol.)	0.5	gm.
Orange G	0.25	gm.
Fast Green FCF	0.25	gm.
phosphotungstic acid c.p.	0.075	gm.
phosphomolybdic acid c.p.	0.5	gm.
glacial acetic acid	1.0	cc.

Microscopic appearance. Shorr describes the results as follows.

It provides a sharp differentiation between cornified and non-cornified elements. The former stain a brilliant orange-red; the latter take on a green stain which is deeper in the younger cells, and paler in those more advanced. The staining is delicate and reveals cytoplasmic and nuclear details very clearly. Other constituents, such as leucocytes, erythrocytes, bacteria and spermatozoa, are satisfactorily differentiated.

Fuchsin Stain (Salmon and Frank, 5; Geist and Salmon, 6:)

Technic. The procedure is described by the authors (6).

A speculum is inserted into the vagina and the vaginal mucous membrane and cervix inspected to exclude the presence of any infection. A small amount of the vaginal secretion is aspirated from the surface of the posterior blade of the speculum with a small glass pipette. The secretion is diluted with a little normal saline, spread on a glass slide, allowed to dry in the air and stained for one minute with fuchsin; the smear is then washed with tap water and is ready for examination.

Reagent: 1. Fuchsin ..... 3.0 gm.
Alcohol 95 to 100% ..... 100.0 cc.
2. Alcoholic fuchsin (1) ..... 12.0 cc.
Distilled water ..... 100.0 cc.

Microscopic appearance. Estrogen deficiency is recognized by the presence of small, round or oval epithelial cells with large, darkly staining nuclei (atrophy cells). The normal smear consists of large, flat, clearly outlined, squamous cells with small, deeply staining nuclei. Details of the smear types observed in the menopause and following estrogen therapy, as described by Geist and Salmon, are included below in the discussion of vaginal smears in the menopause.

Modified Best's Carmine Stain (Salmon, Walter, and Ceist, 7)

Technic. The authors write:

Patients are instructed to take a plain water douche the evening the smears are taken. A speculum is inserted into the vagina and the cervix and fornices are wiped clean. The speculum is then withdrawn, at the same time sweeping off the secretion from the anterior wall with the posterior blade of the speculum. The secretion on the blade is then diluted with an equal part of normal saline and spread on a glass slide. Fixation is obtained by drying in air. A modification of the Best's carmine stain (which is used in the Department of Pathology of the Mt. Sinai Hospital to demonstrate glycogen in tissues) was adapted for the smears.

1. Stain with hematoxylin for 5 min.
2. Rinse in cold water.
3. Stain with carmine solution for 15 min.
4. Differentiate in
   Absolute ethyl alcohol, 16 cc.
   Absolute methyl alcohol, 8 cc.
   Distilled water, 20 cc.
   Immerse 4–5 times.
5. Absolute alcohol—2 immersions.
6. Xylol—2 immersions.
7. Mount with balsam.

Microscopic appearance. The appearance of the preparation is described by the authors.

The glycogen appears as deep red granules in the cytoplasm. In some cells the granules are coarse, while in others they are so fine that their cytoplasm is diffusely pink. The nuclei stain blue. This stain also reveals distinctly the morphologic characteristics of the cellular elements of the smear. . . . Smears taken throughout the menstrual cycle (except during the period of actual menstruation) in women with regular cycles show slight variations in glycogen content which are difficult to interpret. In some women the smears are uniformly lower in glycogen content than in others. This phase of the problem will be reported on later.

Iodine Vapor Stain (Mack, 8)

Technic. 1. *Preparation of smears.* A cotton applicator is inserted into the vagina and twirled lightly (one complete rotation) against the vaginal wall. The cotton end of the applicator is then rolled lengthwise over the surface of a clean glass slide. By rolling, rather than rubbing, a uniformly thin film of cells, with minimal clumping and cell distortion, results. The film dries almost immediately and may be stained at once.

2. *Staining of smears.* Staining is accomplished simply by laying the slide, face down, over a shallow dish containing a small amount of Lugol's solution. Iodine vapors which arise

insensibly from the solution suffice to stain the glycogen-containing cells in two or three minutes. Microscopic examination may be carried out immediately. Although such stains fade in 24–28 hours, re-staining (by the same method) may be carried out repeatedly if later examinations are desired.

Microscopic appearance. Vaginal smears with normal glycogen content are recognizable even macroscopically by the great preponderance of large, flat, polygonal cells with rich brown or violet-brown cytoplasm. The unstained nuclei (recognizable as small, translucent, round bodies) are located in the central portions of the cells. Varying numbers of smaller and more rounded cells taking a deep brown color are also found with some degree of regularity. In contrast to the iodophilic brown cells, a large number of non-glycogenic, unstained or lightly stained lemon-yellow cells of irregular size and shape and poorly defined borders, make up the balance of the normal picture. The latter are the totally cornified squamous cells from the surface of the mucous membrane.... For practical purposes, with this method identification and classification of cell types is unnecessary. The vaginal smear of the normal cyclic woman is recognizable by the preponderance of deeply and uniformly stained brown iodophilic cells.

Smears taken from women during various stages of the menopause as well as from young children prior to puberty are distinguishable macroscopically by the absence of the rich brown color of the stained smear. Such slides have a lemon-yellow, buff, brownish-yellow, or light brown appearance depending upon the degree of glycogen poverty. In extreme grades of vaginal atrophy, as in very young children, or in advanced stages of the menopause, smears consist almost entirely of leucocytes, amorphous debris, and varying numbers of yellow superficial squamous cells. In less advanced stages of ovarian hypo-function (as in earlier phases of the menopause and in pre-pubescent children) minimal glycogen content is discernible by the presence of mottled cells, i.e. cells containing small, irregularly distributed brown deposits at the cell margins and in the cytoplasm. The brown color rarely involves the entire cell as in sexual maturity. The cells in glycopenic smears are generally smaller in size and more rounded in form and have their origin in the deeper layers of the epithelium. Unstained, irregularly shaped cells, typical of the superficial cornified layer are present in abundance. They too appear to be smaller than in normal smears.

As is evident from the foregoing, the original staining methods of Papanicolaou and of Shorr are complicated procedures practicable chiefly for the use of research workers with skilled technical assistance. Shorr's 'single differential stain' seems to provide fewer technical difficulties. The fuchsin and the iodine vapor stains are best adapted for clinical investigations with limited laboratory facilities. The iodine vapor method depends upon a specific color reaction for glycogen alone, places less dependence than other technics upon the cytologic features of the smear, and, hence, requires less microscopic skill and experience.

VAGINAL SMEARS IN THE NORMAL MENSTRUAL CYCLE

Papanicolaou's exhaustive study to date remains the only detailed description yet offered of the normal cycle as revealed in vaginal smears. Briefly summarized, these changes in a typical 28-day cycle are as follows.

Menstrual phase (1–7 days). Erythrocytes, fibrin, bacteria and squamous cells of the superficial or intermediate types; large numbers of leucocytes appear at the end of this period as the erythrocytes vanish.

Follicular or copulative phase (8–12 days). Leucopenia, increase in mucus. The epithelial cells are of the superficial or intermediate types with an increasing tendency to cornification; the cornified cells are usually nucleated.

Ovulative stage (12–13 days, usually). Sudden increase in leucocytes and decrease of mucus. Nucleated cornified cells abundant. Return of erythrocytes indicates ovulatory bleeding.

Post-ovulative or proliferative phase (13–17 days). Variable proportions of leucocytes and cornified cells.

Secretory or premenstrual phase (18–28 days). Abundance of cells and leucocytes. Most cells of superficial type with larger nuclei; decrease in numbers of nucleated cornified cells; rich mucus consistency. With approaching menstruation, erythrocytes, and cell plasmolysis and fragmentation produce 'a pronounced irregularity in the structures of the smear.'

For full details of the cytologic variations occurring in the normal vaginal cycle the reader is referred to Papanicolaou's publication. A careful study of the text of this exhaustive report leaves little doubt as to the probable existence of a histologic cycle as has also been shown (notably by Dierks) by histologic methods. Full accord as to details and extent of these changes has, however, not been reached among investigators of this subject. Hence, until more extensive studies of these cyclic alterations manifested in vaginal smears completely substantiate Papanicolaou's descriptions, his findings must be taken with some reservation. It is obvious, from the bizarre and varied cytologic patterns described, that more than ordinary microscopic skill and experience is necessary to conduct such investigations. Accordingly, it seems evident that the vaginal smear method for investigating normal vaginal physiology is the province solely of the expert histologist.

VAGINAL SMEARS IN CASES OF OVARIAN FAILURE

Menopause

In contrast to the cyclic changes of the vaginal epithelium, which are slight in degree and difficult to interpret, the cytologic characteristics of the atrophic mucosal smear are

striking and more easily recognized, especially in advanced senility and following castration. In advanced atrophy, smears consist of numerous compact, round or oval cells which stain deeply and contain large round or oval, well-preserved nuclei. These are known as 'deep cells' since they are derived from the deeper layers of the vagina; large superficial cells from the uppermost layers are present in small numbers. Leucocytes are also numerous and erythrocytes are frequently found. Smears of this type offer no great difficulties of interpretation to the casual microscopist.

When atrophy of the vagina is less pronounced, however, smears display a high degree of variability. Superficial cells are more numerous, while deep cells may vary in number from great abundance to complete absence. In addition, the cells show an absence of uniformity of outline and structure, they tend to form irregular clumps, and show poor preservation of cytoplasm and nuclei. The presence of large numbers of bacteria gives to the smear a 'dirty' appearance. Papanicolaou and Shorr attribute the great variability in part to the spontaneous re-appearance of rhythmic, cyclic changes. They describe 6 prevailing types of postmenopausal smears: *a*) The menopausal atrophic type; *b*) the intermediate type; *c*) the mucous type, *d*) the premenstrual type; *e*) the bacillus vaginalis type; *f*) the pseudoleucopenic type. The reader is referred to their publication (2) for complete details of this classification.

A more simple classification of the postmenopausal smear was suggested by Salmon and Frank (5) and Geist and Salmon (6); the latter explain the wide variations in degrees of vaginal atrophy on the basis of the varying tempo of the regressive process and continued estrogen elaboration after cessation of menstruation. Geist and Salmon's classification of the various degrees of estrogen deficiency, as determined by morphologic characteristics of smears stained by the fuchsin method, follows.

Reaction I, advanced estrogen deficiency. The characteristic features of this type of smear are the complete absence of squamous epithelial cells and the presence of small, round or oval epithelial cells with rather large, darkly-staining nuclei ('atrophy cells'). These are the cells which Papanicolaou and Shorr have described as 'deep cells.' Leucocytes and erythrocytes are present in varying numbers.

In some smears the epithelial cells are few in number and associated with large numbers of leucocytes; in others, particularly in very old women, the epithelial cells are very small, few in number and are associated with a scattering of leucocytes and erythrocytes.

Reaction II, moderate degree of estrogen deficiency. There is a variable number of large epithelial cells, many of which are irregular in shape. The nuclei are relatively large. Interspersed among these cells is a varying number of atrophy cells and leucocytes. The relative proportion of the large epithelial cells to the atrophy cells is variable. What distinguishes this smear from that of *Reaction I* and *III* is the association of the atrophy cells with the larger epithelial cells.

Reaction III, slight degree of estrogen deficiency. Predominance of rather large, irregular epithelial cells is the striking feature of this smear. The cells vary in size and shape, their edges are somewhat irregular and frequently indistinct in outline. They frequently occur in clumps; a few atrophy cells may be present.

Reaction IV. The smear consists of large, flat, clearly outlined, squamous epithelial cells with small, deeply-staining nuclei. These cells are larger, more clear-cut, and the nuclei relatively smaller than those in the *Reaction III* type of smear. No atrophy cells and usually no leucocytes are seen.

A simple system of grading these varying degrees of estrogen deficiency on the basis of glycogen content, rather than upon morphology of the cellular elements, was described by Mack and Ale (9) utilizing the iodine-vapor staining technic.

Grade I. Complete glycopenia. Smears of this type contain only small yellow cells of varying sizes and shapes and large amounts of amorphous cellular debris. In extreme grades there is marked paucity of epithelial elements.

Grade II. This grade of smear is marked by a greater abundance of epithelial elements than in *Grade I.* Iodine vapor staining depicts glycogen in irregular brown deposits at the cell margins or scattered irregularly throughout the cytoplasm ('mottled cells'). Diffusely stained brown cells, usually of the small, round variety (deep cells; atrophy cells) may also be present in small numbers. Many glycopenic yellow cells are also found.

Grade III. A further increase in cell numbers is evident in this grade as compared to the preceding. The cells are larger and more regular. The diffusely stained cytoplasm has a rich brown color. Non-iodophilic yellow cells are also present in abundance.

Grade IV. This grade of smear is easily recognized by the presence, almost exclusively, of large, flat, deeply stained brown iodophilic cells present singly or in large clumps. *Grade IV* represents maximal estrogenic effect and corresponds to the smear of the normal follicular phase.

A somewhat similar scheme, based on proportions of glycogen-containing and glycogen-deficient cells in smears stained by iodine vapors, was proposed by Willson and Goforth (10).

1+ only an occasional glycogen-containing cell
2+ less than one-third of the cells contain glycogen
3+ from one-third to two-thirds of the cells contain glycogen
4+ more than two-thirds of the cells contain glycogen

Discussion. As is evident from the foregoing discussion, the variable tempo of regressive changes in the vagina and the persistence of high degrees of estrogen activity from non-ovarian or extrinsic sources produce great variability in the appearance of the postmenopausal vaginal smear, both in its cytology and its glycogen content. This has been shown abundantly in smear studies by Papanicolaou and Shorr (2), Salmon and Frank (3), Geist and Salmon (6), Bernstein and Feresten (11), McLaren (12), Mack and Ale (9), and Mack (13). Evidence of postmenopausal estrogen elaboration has also been demonstrated from estrogenic titers in the blood and urine of climacteric women by Robson and his associates; Frank, Goldberger and Salmon; Schlossberg and Durruty; and by Fluhmann (14).

While Papanicolaou and Shorr have insisted that 'the menopausal type of smear is invariably present after menopause whether or not symptoms exist' it is evident that such smear pictures are not always clearly defined nor readily recognized. Hence, clear-cut evidence of ovarian failure is not invariably demonstrable during the symptom-producing phases of the menopause and the diagnostic value of smears is highly debatable and necessarily limited. Ottaway's conclusion (15) gives a good appraisal.

> In spite of promising experimental data, it is obvious from our observations that vaginal smears are far from infallible as clinical objective evidence in estrogenic insufficiency of women. For example, the fact that no estrogenic deficiency was revealed by smears in 4 cases with symptoms which followed castration shows lack of reliability in absolute diagnosis, although the method could well be of some use as confirmatory evidence in vague cases.

Amenorrhea and Other Disorders of the Menstrual Cycle

Papanicolaou and Shorr also described the vaginal cytology in primary and secondary amenorrhea. Smear types identical with those they described for the menopause were found.

> *The menopausal atrophic type* is characteristic of cases of primary amenorrhea, being associated with an infantile uterus. The deep cells prevail in this type. In secondary amenorrheas, all other types described above are found. . . . The resemblance of the smears in these two conditions (i.e., menopause and amenorrhea), the similarity in their response to the follicular hormone, and the existence of postmenopausal cyclic changes, tend to break down the sharp distinctions generally held to exist between them. From the morphologic standpoint it is frequently hard to classify cases in one group or the other.

In a later publication (16) they describe the vaginal smears in a case of secondary amenorrhea of the stationary or 'fixed' atrophic type. More recently, Papanicolaou spoke of the 'atrophic type of smear, usually found in primary amenorrhea' and a 'crowded type of smear, usually found in primary amenorrhea.' (17).

Moulton (18) has reported the results of the study of vaginal smears in three patients with anorexia nervosa who had disturbances of the menstrual cycle. In one instance, smears of the follicular type with irregular cyclic changes were interpreted as

> evidence of moderate continuous estrogen activity, as the cells were of the intermediate type rather than of the basal type, and were distinct, well-formed, and partly cornified. The percentage of cornification was taken as the best and only quantitative measure of the level of estrogen activity, and cyclic increases in this were found to coincide with, or just precede vomiting attacks. The quiescent level of cornification between attacks was 2 to 10 per cent, with definite increase during attacks to 25 to 40 per cent. . . . They were thought to be of significant magnitude because they consisted of at least 20 per cent increase in cornification, while the margin of error in estimating each smear was about 5 per cent as shown by rechecking. . . . Smears were also taken three times a week on *case 3* for five months, but no cyclic changes were found, the smears being of the atrophic type, described as being typical of primary amenorrhea and severe secondary amenorrheas. . . . Vaginal smears were useful in this study for research rather than for therapy.

Ripley and Papanicolaou (17) also reported study of vaginal smears in menstrual disorders in 31 women with schizophrenia, depression and elation. Their investigations showed 'well expressed follicular reactions' in the presence of normal cycles, whereas retarded menstruation was attended by the 'relatively frequent occurrence' of a delay in the appearance of the follicular phase. In most patients with abnormally long intervals the follicular reaction in smears was either poorly expressed or absent. Such atypical follicular reactions were described

as ones in which 'cornified cells of the follicular type are intermixed with smaller, round or oval deep cells, indicative of a subnormal estrogenic secretion.' Short cycles were rarely observed in this study, although in one instance of a 19-day interval the smear was marked by the persistence of numerous cornified cells during the premenstrual period, whereas in the normal premenstrual stage their numbers decrease gradually. Scant menstrual bleeding was observed in instances in which 'the follicular reaction was poorly expressed' and the interval was lengthened.

Discussion. While the value of the vaginal smear method in clinical research of cyclic disturbances has been shown in the examples cited above, there is little evidence at present to indicate its usefulness in gynecologic practice. Readily distinguishable vaginal atrophy indicative of extensive ovarian deficiency appears to be characteristic of certain types of primary amenorrhea; the highly variable pictures seen in the vaginal smears of cases of secondary amenorrheas and of irregularities of the menstrual interval make this procedure of questionable value. Campbell and Sevringhaus (19) claim practical importance of vaginal smears, along with endometrial biopsy and pregnandiol determinations, for accurate diagnosis and management of amenorrhea. The lack of other confirmatory reports from clinical workers would appear to indicate the limited value of the smear method in this field.

Failure of Ovulation

Papanicolaou's description of the 'ovulative smear' suggested the use of this method for the detection of ovulation or the recognition of anovulatory cycles in investigations of functional sterility. The ovulative stage which follows upon the leucopenic follicular phase is recognized, according to Papanicolaou, by the sudden appearance of leucocytes and a few erythrocytes. After accomplished ovulation, there follows the luteal phase of the cycle the smear for which is described as consisting of cells which are folded, clumped, fragmented, and associated with many leucocytes; prior to ovulation, the cells are intact, discrete, largely cornified and unaccompanied by leucocytes.

Use of the smear method for the detection of ovulation or ovulatory failure has been advocated chiefly by Rubenstein (20, 21) who combines the cytologic changes described above with determinations of basal rectal temperature for this purpose. Depending upon evidence of a sudden rise of basal rectal temperature and the ovulatory changes in vaginal smears, Rubenstein reported a high incidence of anovulatory menstruation in cases of sterility. He pointed out that the presence of infection or chronic irritation may, however, invalidate the interpretation of both smears and temperature readings. Zuck and Duncan had previously relied upon a rise both in vaginal pH and rectal temperature to postulate the occurrence of ovulation (22).

In a recent critical study of the smear method for detecting ovulation, Krohn, Harris and Hechter (23) indicate that numerous factors complicate the evaluation of ovarian function by this means. They showed that *a*) artifact peaks of desquamation frequently occur without a corresponding drop in cornification and these do not signify ovulation, and *b*), persistently high levels of estrogen may induce a desquamation similar to that produced by progesterone during the luteal phase of the cycle. They believe, however, that proper interpretation of daily vaginal smears will 'in most cases' indicate whether or not the patient has ovulated, although no explanation for 'artifact desquamations' is offered. They supplement observations on luteal activity by vaginal smear studies with determinations of urinary pregnandiol.

No confirmatory evidence of the value of vaginal smears for the detection of ovulation has as yet been produced.

Senile Atrophic Vaginitis

The use of vaginal smears for the recognition of postmenopausal vaginitis resulting from estrogen deficiency and the control of specific therapy of this condition was reported by Ottaway (15). The limited value of smears for this purpose is summarized in his conclusion: 'In the diagnosis of senile vaginitis the examination of vaginal smears could be of considerable value in ruling out the suspected but not true cases.'

VAGINAL SMEARS FOR EVALUATION OF THERAPY

The striking and easily demonstrated transformation of the atrophic vaginal smear by the administration of estrogens and the definite induction of atrophic changes in the normal and estrogen-stimulated mucosa by androgens suggested the usefulness of the smear method as a means for evaluating the success of therapy using the sex sterols.

Estrogen therapy. Papanicolaou and Shorr (2) were the first to advocate the use of vaginal smears for objective determinations of the effectiveness of estrogen therapy independent of symptomatic response. They suggested the attainment of the follicular phase as the therapeutic goal.

> The fact that symptomatic relief may occur before the final smear change raises the question whether the optimum state is reached when symptoms are relieved or when the follicular phase is induced. Analogies are numerous with other deficiency states, in which objective as well as subjective criteria are available. The relief of the symptoms of myxedema at lower than normal basal metabolic levels, and the abolition of tetany, with the blood calcium and phosphorus still far from normal, are examples. Such partial changes are welcome from the standpoint of the patient's comfort. But it is generally recognized that they are less desirable than the complete restoration of the physiologic state prevailing with the normal function of the hormones involved. It is for this reason as well as the other considerations mentioned above, that we are inclined to consider the induction of the follicular phase as the more reliable standard in the treatment of the menopause syndrome.

Papanicolaou and Shorr further utilized the attainment of the follicular phase as a therapeutic test to distinguish between menopausal and pseudo-menopausal symptoms, since the latter 'will persist after complete smear changes are obtained.'

Acceptance of the vaginal smear method as an important or necessary adjunct to the evaluation of estrogen therapy has been recorded by several investigators. Campbell and Sevringhaus (19) consider smears, along with endometrial biopsies and pregnandiol determinations, essential for accuracy in diagnosis and appraisal of therapy. Smith (24), using smears as diagnostic measures and as guides to therapy found that 'alterations in the smear practically always parallel symptomatic improvement.' Schneider (25) states that 'vaginal smears provide an efficient and accurate index to physiologic action of estrogen and although of value in the individual case are not essential routinely.' Ottaway (15) recognized the occasional usefulness of smears in the treatment of senile vaginitis as 'a means of gauging the dosage of estrogenic substance to attain the normal *type IV* smear necessary for cure.' Simpson and Mason (26) employed vaginal smears to demonstrate the effectiveness of vitamin A therapy in senile vaginitis which they attribute to deficiency of vitamin A as well as of ovarian hormones.

Other investigators have been less enthusiastic and even critical of the value of vaginal smears in therapeutic appraisals. Werner and his associates (27) wrote:

> It is quite evident from the present study that changes in the vaginal secretion are a much less delicate index of the effectiveness of estrogenic material than is an examination of the uterine mucosa secured by curettage. . . . It is also quite obvious that symptomatic relief can be secured in dosages that are too small to produce definite changes in the vaginal secretion, since the group of subjects receiving the smallest dosage apparently secured as much relief of symptoms as those who received larger doses. In view of these facts we feel that the question may legitimately be raised whether the objective of theelin medication in castrate and menopausal cases should be pushed to the point of full follicular phase of the menstrual cycle, as suggested by Papanicolaou.

Ottaway concluded (15):

> In spite of promising experimental data, it is obvious from our observations that vaginal smears are far from infallible as clinical objective evidence in estrogenic insufficiency of women. For example, the fact that no estrogenic deficiency was revealed by smears in 4 cases with symptoms which followed castration shows lack of reliability in absolute diagnosis, although the method could well be of some use as confirmatory evidence in vague cases. In treatment of the so-called menopausal syndrome, progression of smears toward an estrogenic sufficiency type, showed a general relationship to improvement in symptoms, but with so many exceptions as to be of only slight value in determining dosage for relief.

The same viewpoint was expressed by Bennett and TeLinde (28) who state:

> We do not feel that it is necessary for the practitioner who is treating menopausal symptoms to follow the menopausal smears. In fact, to be guided entirely by the smears would in some instances be misleading.

Androgen therapy in women. Vaginal smear studies by Papanicolaou, Shorr, and Ripley (29), as well as those of other investigators, have demonstrated atrophic changes following the administration of testosterone propionate. While these reports suggest the usefulness of vaginal smears as an index of the effect of androgens upon women, no clinical application of this method has as yet been made in the management of gynecologic disorders with male hormone preparations.

VAGINAL SMEARS FOR HORMONE ASSAYS

Confirmation of the observations that the atrophic vaginal smear can be readily transformed into one resembling the follicular phase of the normal cycle has resulted in numerous attempts to evaluate the effectiveness of various hormone preparations in human subjects by this method. The hormones tested by this means now include, not only the various natural and synthetic estrogens, but also progesterone, androgens and gonadotropins. Only a few of the more representative articles of the voluminous literature are cited in this appraisal of the vaginal smear method.

Estrogens. Potency of the various products has been judged by their ability to transform the atrophic vaginal smear of menopausal or castrated subjects into one characteristic for the cytologically normal follicular phase. This evidence of estrogenic efficiency has been shown for estrone, α-estradiol benzoate, α-estradiol dipropionate, ethinyl estradiol and diethylstilbestrol. The effectiveness of these preparations has been shown also by oral, parenteral, sublingual, vaginal routes of administration and by subcutaneous implantation and inunction. The cytologic criteria of Papanicolaou and Shorr were made the basis of most determinations. The glycogen index was employed by Mack (8), Mack and Ale (9) in studies of oral, vaginal and parenteral administrations of estrone. Willson and Goforth (10) tested diethylstilbestrol by this method and observed that an increase in vaginal glycogen after treatment could be observed before changes in cell type were detectable. Herrnberger and Horstmann (30) have assayed estradiol benzoate by means of the glycogen index of smears stained by the complicated Best's carmine method. Their studies were made upon young children. Further investigations are necessary to determine whether the technically simple glycogen methods or the more complex procedures based upon cytologic criteria are best suited for estrogen assays in the human subject.

Stoddard and Metzger (31) have employed the vaginal smear method to determine the comparative potencies in women of orally administered diethylstilbestrol, and α-estradiol benzoate and estrone by intramuscular injection. The effectiveness of these substances was compared by determining the minimum amount of each which was necessary to produce a mid-interval type of smear response in the middle 75 per cent of each group of menopausal subjects on the day following cessation of a 10-day period of treatment. When assayed by this procedure (which follows closely the manner of assay of estrogens in laboratory animals) they determined that 1.0 mg. of estrone (intramuscularly), 0.42 mg. of α-estradiol benzoate (intramuscularly) and 0.5 mg. of diethylstilbestrol (orally) are comparable dosages in the human subject. Ferin (32) proposed a similar approach to determine human units for estrone estradiol and stilbestrol from vaginal smear and endometrial responses in castrated women. Mack and Ale (9) have compared the efficiency of orally and parenterally administered estrone on the basis of glycogen responses to equal weighed amounts given to menopausal subjects having advanced vaginal atrophy. Further large scale investigations on human subjects are necessary to determine whether this method will provide reliable data for clinical use. The widely divergent results of animal assays indicate the need for a trustworthy means of determining human responses and requirements. The published reports of estrogen determinations based upon vaginal smear responses in human subjects indicate a promising approach toward fulfillment of this need.

Progesterone. The effect of progesterone on vaginal smears was studied by Shorr (33). His report indicates that the administration of progesterone and estrogen reproduces, more fully than estrogen alone, the physiologic picture of the second half of the menstrual cycle described by Papanicolaou. No other study of this type appears in the available literature. Hartman and Speert's study (34) on unprimed castrated rhesus monkeys indicates that growth and cornification of the vaginal mucosa results after progesterone administration and suggests that there is little difference in the vaginal action of estrin and progesterone.

Gonadotropins. Shorr and Papanicolaou (16) and Campbell and Sevringhaus (19) appear to be the only investigators who have attempted to judge the efficacy of gonadotropic hormone preparations by evidence from vaginal smears

of increased levels of estrogenic response. Shorr's patient showed typical estrogenic smear changes after the first course of therapy with gonadotropic hormone which resulted in menstruation; the patient, thereafter, remained refractory to further treatment as was shown by continued 'fixed' atrophic smears. Campbell and Sevringhaus consider gonadotropic hormone therapy useless if vaginal smear changes (as well as endometrial patterns and pregnandiol determinations) do not indicate that full ovarian activity has been attained.

Androgens. The anti-estrogenic action of androgens has been demonstrated in vaginal smears by the production of atrophy in the normal and estrogen-stimulated mucosa of women. Salmon, Walter and Geist (7) have also shown that testosterone brings about a disappearance of glycogen from the cells of the vaginal epithelium. The use of the vaginal smear method for evaluating androgen preparations was suggested by Shorr, Papanicolaou and Ripley (35):

> Testosterone propionate inhibited the menstrual cycle of a young menstruating woman. Menstruation was resumed spontaneously 28 days after cessation of therapy. Examination of the vaginal smears was made during the various phases of the menstrual cycle. With the administration of the male hormone, a transition to an atrophic menopausal type of smear took place. With the cessation of therapy the smears returned to normal. These definite changes in the vaginal secretion offer a simple and reliable method for evaluating the effect of the male hormone in women.

This has been abundantly confirmed in trials of testosterone propionate by Rothermich (37); Geist, Salmon, Gaines and Walter (38); Rothermich, Postle and Foltz (39); and others. In an early publication in 1937 Salmon (40) reported a contrary observation in a 46-year-old castrate after injection of 400 mg. of testosterone propionate.

> It would appear from this study that testosterone propionate when given in adequate dosage can inhibit the gonadotropic hyperactivity of the hypophysis in the female castrate and also produce a full estrogenic effect on the vaginal mucous membrane in a female castrate—thus paralleling two phases of biologic activity of the estrogenic substances.

This report appears to be the only one to contradict the antigynecogenic action of testosterone upon the human vagina.

Gaines, Geist and Salmon (41) noted no suppressive action upon the vaginal mucosa from the administration of various dosages of pregneninolene and concluded, thereby, that its androgenic effect was *nil* or negligible.

CONCLUSIONS

The vaginal smear method has yielded a wealth of scientific information concerning the endocrine physiology of the menstrual cycle. In scientific investigations of the menopause and other conditions of ovarian failure, smears have also provided valuable objective evidence of functional derangement. As a method of diagnosis of ovarian dysfunction or failure in clinical practice the vaginal smear method has very limited usefulness because of: *a*) difficulties of interpreting the bizarre and highly variable histologic smear patterns, and *b*) the frequency of normal smears in the presence of signs and symptoms of ovarian failure.

As a clinical method of determining the effectiveness of estrogen therapy, the vaginal smear technic may provide objective evidence of therapeutic results in limited instances in which vaginal atrophy accompanies other signs or symptoms of ovarian failure.

The most promising field of usefulness of the vaginal smear method is its application as a means of estrogen and androgen assay in the human female.

Until the technic of preparation and the criteria of interpretation of vaginal smears are simplified, use of the procedure will be restricted to research studies.

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COMMUNICATION TO EDITOR

A NEW METHOD FOR STAINING SPERMATOZOA

It is only during the past 20 years that clinicians interested in the study of male infertility have realized the importance of investigating the physiology and morphology of spermatozoa. Previously the presence of live spermatozoa was accepted as conclusive evidence of male fertility. Many workers are now stressing the importance, not only of numbers, motility, biochemistry and endurance, but also of morphology. Moench[1] was the first to apply statistical methods to the study of the morphology of human semen as related to sterility. He found that in specimens from normally fertile human males the number of abnormal head forms never exceeded 20 per cent of the total count. As values very close to this have been obtained by almost all of the many later workers[2,3,4] a ratio of 20 per cent abnormal forms has become more or less accepted as the region of demarcation between normal and lowered fertility.

The range of abnormalities in sperm is very wide and includes malformations of head, neck and tail, those of the head being most important and numerous. For this reason, various methods have been devised to stain differentially the head, neck and tail. In most cases the procedure is time-consuming and involves so many steps that each slide must be handled individually. In view of these disadvantages, our object was to find a differential stain which could be used successfully even by the inexperienced, and which would require a minimum of time and effort. We believe that this has been achieved.

The stain, a modification of the Pappenheim-Saathoff formula[5] has been used for smears of gonococci,[6] but to our knowledge has never been recommended for the staining of sperm. With its use as outlined below the sperm head is colored blue-green, the acrosome lighter than the posterior part of the head; neck and tail are pink to lavender, less distinct than the head but clearly visible. We believe there may be a tendency for abnormal forms to stain more darkly than the normal.

FORMULA AND PREPARATION

methyl green, dye content 60%	1.0 gm.
pyronine, bluish, certified	0.2 gm.
methyl alcohol, absolute	10.0 cc.
phenol, 2% aqueous solution	100.0 cc.
glycerol, c.p.	20.0 cc.

Dissolve the dyes in the alcohol and phenol by intermittent shaking about 2 hours each day for 2 days, using a mechanical shaker. Filter and add the glycerin. Refilter into dropping bottle as the supply is needed.

An alternate formula follows:

methyl green, dye content 60%	1.0 gm.
pyronine, bluish, certified	0.25 gm.
elcohol, 95%	5.0 cc.
glycerol, c.p.	20.0 cc.
phenol, 2% aqueous solution	100.0 cc.

Heat solvents to about 60° C. Rub dyes together and add to solvents. Agitate until dyes are dissolved. The solution should be allowed to stand for an hour or more before filtering.

PROCEDURE

1. Prepare a smear on a clean dry slide. Dry in air.
2. Fix in a solution of 1 part 95 per cent alcohol, 1 part ether, and dry in air; or fix by heat.
3. Flood the slide with stain. If desired, heat the slide until steam rises from the stain. This latter step is not necessary but results in better staining of some cellular elements. Let stand 5 minutes.
4. Wash with tap water.
5. Dry and examine with oil immersion lens.

Received for publication December 28, 1942.

[1] Moench, G. L.: Sperm morphology in relation to fertility. *Am. J. Obst. & Gynec.*, 22: 199. 1931.

[2] Hotchkiss, R. S., E. L. Brunner and P. Grenley: Semen analyses of two hundred fertile men. *Am. J. M. Sc.*, 196: 362. 1938.

[3] Stiasny, H.: Method of examination of sperm of fertile and sterile males with special reference to morphology of spermatozoa. *Zentralbl. f. Gynäk.* 61: 2051, 1937.

[4] Pollak, O. J., and C. A. Joel: Sperm examination according to the present state of research. *J. Am. Med. Assoc.* 133: 395. 1939.

[5] Conn, H. J. Biological stains. Commission on standardization of biological stains. Geneva, 1929.

[6] Walton, S. T.: Quick and reliable method for staining gonococcus smears. *J. Lab. & Clin. Med.* 24: 1308. 1939.

This method has been used in semen studies of over 350 cases, chiefly cases from private practice and the results have been similar to those obtained with other more complicated differential stains.

SUMMARY

Formula and procedure are given for a simple rapid differential stain for human spermatozoa. Staining results are briefly described. The method has been used in the study of over 350 cases of suspected low fertility in the male.

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EDITORIALS

HORMONE THERAPY AND BENIGN HYPERTROPHY OF THE PROSTATE

THE POSSIBILITY of endocrine therapy for benign hypertrophy of the prostate is of considerable interest to the physician. Reports of successful treatment of this condition have appeared, and the doctor is urged to try an estrogen or an androgen, frequently by the patient himself, more often by the ubiquitous detail man.

It may be well to examine, for the benefit of the practicing physician, some of the experimental evidence which led to attempts at endocrine therapy for nodular prostatic hypertrophy.

The response of the prostate to many varieties and combinations of hormones has been investigated in many animals. In rodents, gonadotropic hormones given to intact animals or androgens administered to immature or castrated animals causes growth of both parenchyma and stroma of the compound prostate gland. Estrogens affect mainly the utricle, and with prolonged treatment cause a squamous cell metaplasia, not only in the utricle but also in the collecting ducts of the prostatic acini.

The dog is particularly interesting in that a high incidence of cystic glandular hyperplasia of the prostate occurs spontaneously with ageing. Although there is a great increase in mass of the prostate in such aged dogs, there is no formation of fibromuscular nodules, and so far as appears in the records, little or no urinary retention.

In dogs reported by de Jongh and Kok,[1] estrogen caused a cystic dilatation of prostatic glands and the utriculus with squamous metaplasia and desquamation. There may have been some suggestion of fibromuscular overgrowth but in no instance has this been localized or nodular. Huggins[2] and his associates demonstrated prostatic enlargement in the dog after estrogen treatment. The gland was markedly enlarged, but with cystic dilatation of the prostatic acini only, and without fibrosis, nodule formation or urinary retention.

Benign prostatic hypertrophy has not been reported for any species of monkey or any subhuman primate. In a report of an old rhesus monkey with a well-developed adenocarcinoma of the prostate, there was no evidence of concurrent benign prostatic hypertrophy.

Young preadolescent castrated male monkeys have been treated with estrone, with testosterone propionate and with combinations of the two for brief periods.

In Zuckerman's series[3] two monkeys were treated with small doses (10 μg. and 100 μg., respectively) of estrone for a year. In these two, the main effects of the estrone consisted in an increase in size of the fibromuscular stroma of that portion of the prostate which forms the bed of the utriculus.

The monkeys which have been treated were young animals. It is possible that further developments would be observed if old animals were used. Fibromyomatous nodular hyperplasia of the human prostate is structurally very similar to fibromyomata of the uterus. Lesions similar to those of the human uterus have been induced in guinea pigs by prolonged estrogen stimulation. No observable lesion has yet been induced in the fibromuscular tissue of the uterus of the rhesus monkey. Treatment of fully mature female monkeys with an estrogen for periods as long as 170 days, produced no such change. Aged monkeys, which had received estradiol crystals or pellets with a total dosage of 825 mg. during 24 months, have likewise shown no fibromyomatous nodules in the uterine musculature.[4]

The possible endocrine participation in benign prostatic hypertrophy has, as yet, received no support from animal experimentation. The prostate may be induced to grow greatly in size, and cystic dilatation of the glands may occur, but

[1] DE JONGH, S. E., AND D. J. KOK: *Acta brev. Neerland.* 177. 1935. Microphotographs of the work of these investigators was published in ZUCKERMAN S. AND GROOME J. R.: *J. Path. & Bact.* 44: 113. 1937.

[2] HUGGINS, C.: Exhibit, Annual Meeting American Medical Association, New York City, 1940.

[3] ZUCKERMAN, S.: Effects of prolonged estrogenic stimulation on the prostate of the rhesus monkey. *J. Anat. 72*: 264. 1938.

[4] ENGLE, E. T., AND C. KRAKOWER. Unpublished.

no fibromuscular lesions have been produced in the male, and urinary retention as a result of such treatment has not been induced.

The excretion of known gonadotropins or steroids by patients with benign prostatic hypertrophy does not differ significantly from that by other patients of similar ages.

The well-known relationship of the testes and their steroids to the growth and maturation of the human prostate, and the suggestive relationship in senile involution of the prostate, make it inadvisable, however, even in the face of negative data to imply that endocrine changes may not be at least a factor in benign nodular hyperplasia. Several papers[5] have been presented showing clinical effects or improvement of patients with this disorder, after treatment with certain steroid hormones.

These papers with their case histories are difficult to evaluate. The patient calls on the physician not because of prostatic hypertrophy, but because of urinary frequency, painful micturition or acute retention. Many manipulative procedures give temporary relief. Prostatism itself may be intermittent, with spontaneous remissions of the disease. Any claims for relief of prostatism, other than surgical, must be scrutinized carefully in the light of the well known 'control series' of patients who refused operation for obstructive prostatism. This notable group reported by Clarke[6] were patients who were observed after refusal of operation for about four years, and in whom some degree of urinary efficiency was maintained by routine procedures. These cases are not reviewed to contrast the degree of satisfaction obtained in surgical as compared with non-operated cases, but should warn against undue optimism regarding 'cures' of prostatism by endocrine therapy.

There may be theoretical grounds for believing that either estrogen or androgen might be effective therapy in prostatism. The difficulties of evaluating the results of such therapy in terms other than 'patient relief' are very great. The tangible evidence of structural improvement is meager or lacking and it is doubtful that remissions or relief of this type of prostatism by endocrine therapy is any greater than has been obtained by other procedures short of surgery.

The brilliant demonstration by Huggins[7] that castration results in physiologic and clinical improvement of patients suffering from adenocarcinoma of the prostate indicates clearly, however, that the door is not closed to further research on the hormonal relations of prostatic physiology and pathology.

Work reported to date has not shown clearly an endocrine rôle in the etiology or the successful therapy of nodular hyperplasia of the prostate.

New interest and activity in investigating the rôle of the liver and the adrenal in the intermediary metabolism of the steroids, and the possible relations of various steroids to problems of collagen formation and fibrosis, all promise that problems of the pathology of the prostate will not be neglected.

For the time being, however, the patient suffering from urinary retention as a result of nodular hyperplasia of the prostate will be afforded greater relief by the surgeon than by the endocrinologist.

E.T.E.

[5] LOWER, W. E., F. C. SCHLUMBERGER AND E. E. FERGUSON: The hormonal treatment of benign enlargement of the prostate. *Surg., Gynec. & Obst.* 71: 354. 1940.

[6] CLARKE, R.: The prostate and the endocrines. *Brit. J. Urol.* 9: 254. 1937.

[7] HUGGINS, C., AND C. V. HODGES: *Cancer Res.* 1: 293. 1941.

ADRENAL-CORTEX EXTRACT AND SHOCK

THE GENERAL picture of functional depression following destruction of the adrenal cortex and that of surgical shock are suggestively similar—a fact that has long been recognized. Many writers have speculated as to the degree to which the manifestations of shock might actually mirror cortical failure. Claims have been made that cortical extract when administered to patients or animals in shock to some extent parallels its brilliant efficacy in counteracting adrenal deficiency. On the other hand, many essentially negative results have been obtained and doubt is growing as to the practical value of adrenal-cortex therapy in the treatment of shock.

Among those who have reported favorable results are workers in the laboratories of the Michael Reese Hospital in Chicago. Here a method was developed for the production of shock by occlusion of the veins in the hind legs of dogs. It was found that this procedure resulted in shock followed by death, usually within a few

hours in 87 per cent of the subjects. Desoxycorticosterone acetate was found to have considerable protective value. More recently Shleser and Asher[1] of the same institution have repeated the work, using, instead of the synthetic preparation, a commercial extract of adrenal-cortex material. It seemed reasonable to hope that its other active components might add to the efficacy of the contained desoxycorticosterone since previous investigators had found that cortin was effective in preventing the occurrence of histamine shock in adrenalectomized animals; it also had a beneficial effect on the reduced blood pressure and the plasma-fluid loss in dogs which had received, intravenously, extracts made from obstructed loops of intestine. More immediately relevant are earlier findings that cortical extract had an ameliorative effect on shock following intestinal trauma in rats, and in the prevention of traumatic shock in dogs.

In Shleser and Asher's new work the veins of one limb of the dog were ligated aseptically. Suspensions of sterile lamp black in normal saline solution were then injected. As criteria of the development of shock, measurements of blood pressure, heart rate, relative blood cell-volume (hematocritic) and limb circumference were recorded at regular intervals postoperatively. To the experimental animals adrenal cortical extract was administered subcutaneously in dosage varying from 17.5 to 39.0 cc.—the average being 23.3 cc. One-half of the total dose was given as a prophylactic 12 hours preceding the operation and the remainder in divided doses over the first 12 hours, following the operation.

Twelve experimental animals were used. Five of these died within 24 hours after the operation and postmortem studies supported the assumption that the deaths had been due to shock. One dog, in addition, died of pneumonia within the first day but appeared not to have developed shock. Thus 50 per cent of the animals survived, as compared with only 13 per cent which, on the basis of previous experiences, was expected.

In the 5 animals which succumbed to shock the affected limb had increased in size about one-third beyond that of its control member. This increased weight amounted to from 3 to 8 per cent of the total body weight and thus represented a loss of plasma fluid equivalent to from a half to three-fourths that of the total blood volume. The treatment with adrenal cortical extract seemed to have been completely ineffective in these 5 animals since they survived no longer than did untreated controls. Among the 6 dogs that survived limb enlargement was slightly less, but occurred after a longer interval than in those that died. In 3 of the 6, transitory fall of blood pressure was noted but in the other 3 such did not occur. Slight hemoconcentration occurred in 3 and no significant change in the other 3 dogs. Among the 6 survivors none showed clinical signs of developing shock.

To what extent the induction of limb-edema induces conditions of true 'shock' may be questioned. The nervous system is obviously spared the bombardment of nociceptive impulses that result from ordinary types of bodily injury. Furthermore, the experimental procedure fails to reproduce the psychic elements that seem to contribute importantly to the development of surgical shock in man. Even so, however, the new work is suggestive and offers encouragement for further investigations. It is quite possible, as the authors suggest, that with greater knowledge of the composition of the adrenal extract and further research on the physiology of its active components still more satisfactory results can be achieved.

Among the questions upon which further evidence is needed is whether adrenal cortical hormone may be effective when given after shock has supervened or whether its effectiveness is limited to instances in which it is used, as in Shleser and Asher's work, prophylactically as well.

Another aspect of the problem is the question as to the optimal number of doses into which the given amount of extract should be divided. It is perhaps relevant that Swingle and co-workers[2] have found that a given amount of plasma used in the treatment of constriction shock was much more effective when given in 5 divided doses than when administered as a single infusion.

R.G.H.

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[1] Shleser, I. H., and R. Asher: Efficacy of adrenal-cortical extract and of paredrine in the prevention of experimental shock following venous occlusion of a limb. *Am J. Physiol.* 138: 1. 1942.

[2] Swingle, W. W., J. W. Remington, W. Kleinberg, V. A. Drill and W. J. Eversole: An experimental study of the tourniquet as a method for inducing circulatory failure in the dog. *Am. J. Physiol.* 138: 156. 1942.

LETTERS

USE OF PITRESSIN TANNATE IN OIL AS A HEMOSTATIC AGENT IN MENORRHAGIA AND METRORRHAGIA

To the Editor:

The use of pitressin tannate in oil as a hemostatic agent in a series of cases of menorrhagia and metrorrhagia has been attended with uniformly good and rapid results. This use of a standard product appears to be new.

Pitressin tannate in oil is a water-insoluble preparation of the pressor and antidiuretic principle of the posterior lobe of the pituitary gland suspended in oil.* Injection of this intramuscularly is followed by slow absorption and prolonged action. It was originally produced for use in diabetes insipidus and has been used successfully in this condition.[1,2,3,4] Because of its prolonged vasoconstricting effect its use in menorrhagia and metrorrhagia or functional uterine bleeding seems logical.

The 10 patients treated included 4 with menometrorrhagia and 6 with metrorrhagia of the premenopausal and menopausal type. Without exception they responded with cessation of the flow within 6 to 72 hours following administration of the first or second dose of 1 cc. in oil, given intramuscularly.

Two of the 4 cases of meno-metrorrhagia were young girls. One had bled for 2 months and the other for 28 days. In each case two injections of the pitressin tannate in oil were sufficient to control the bleeding. In these cases of prolonged bleeding it has been found that two injections of 1 cc. each, given at 3-day intervals a few days before the next period is due, will prevent the menorrhagia.

When the periods are irregular, difficulty will naturally be encountered in estimating the date the next menstruation is due; but in these 4 cases of menometrorrhagia, the injections were begun approximately 20 days after the beginning of the last flow, except in the one case which flowed 2 months. In this individual the injections were given 2 weeks after the flow had been stopped by the administration of pitressin tannate in oil.

In 6 cases of premenopausal and menopausal metrorrhagia, one or two injections of 1 cc. of the pitressin tannate in oil controlled the bleeding. These cases had been classified as functional uterine bleeding. Naturally as in all cases of this type the usual precautions should be taken to rule out organic causes of bleeding. Metrorrhagia due to carcinoma may be temporarily checked by treatment and thus give a false sense of security.

No other form of therapy that I have used for functional uterine bleeding has given such a prompt response. This treatment is also economical, which certainly is in its favor since so much endocrine treatment is expensive.

No untoward results other than a mild headache have been observed. Occasionally stinging and burning at the site of injection were complained of.

There is, of course, the possibility of excessive water retention if the material is given in high dosage or over prolonged periods. The signs and symptoms of excessive water retention are rapid weight increase, headache, listlessness and drowsiness. The dosage should be reduced or treatment suspended in such instances.

So far I have used only the 1-cc. dose, and it is quite possible that smaller doses than this will suffice. However, so few injections have been necessary in the patients thus far observed that there has seemed to be no danger of overdosage. Naturally, good judgment should be used in patients with hypertension, nephritis, and cardiovascular disease.

Greenblatt[5] concludes that the myometrium as well as its vascular supply is of great importance in metrorrhagia. He says that excessive uterine bleeding will occur if the proximal (myometrial) portion of the spiral arterioles fails to constrict or be constricted following the initial extravasation of blood distally. The endometrium seemingly plays a minimal rôle in this type of bleeding. If this is true then the prolonged vasoconstricting effect of pitressin tannate on the myometrial vascular system would help to explain its benefits in meno-metrorrhagia. Control of the bleeding tends to prevent the secondary anemia which so frequently accompanies these conditions.

This report is made only to stress the fact of the prompt control of functional uterine bleeding. Further studies are, of course, necessary. The use of pitressin tannate in oil in other conditions in which prolonged uterine vasoconstriction is desirable suggests itself.

As a preventive of functional uterine bleeding in subsequent periods, the practice of giving the drug a few days before onset of the expected flow seems to warrant further study. So far, in the few cases tried, it

* Parke, Davis & Company.

[1] Greene, J. A., and L. E. January: *Proc. Soc. Exper. Biol. & Med.* 44: 217. 1940.

[2] Stephens, D. J.: *Proc. Soc. Exper. Biol. & Med.* 44: 240. 1940.

[3] Thorn, G. W., and K. E. Stein: *J. Clinical Endocrinology* 1: 680. 1941.

[4] Cady, L. D.: *J. Missouri M. A.* 39: 19. 1942.

[5] Greenblatt, R. B.: *J. Clinical Endocrinology* 2: 345. 1942.

has been successful in preventing excessive flow, the periods usually lasting 4 to 6 days with a moderate amount of bleeding.

Further studies are being made and I hope this report will prompt the use of the drug by others so that its value in functional uterine bleeding will be adequately determined.

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PSYCHOSOMATICS OF MATERNAL BEHAVIOR

TO THE EDITOR:

In a recently published article[1] I reported some of the results of a search for constitutional factors in maternal behavior. Interviews were held with 72 women, nearly all of whom were mothers of children who were treated for various behavior problems. A relationship was found between the habitual number of days of menstrual flow and the strength of the maternal feeling. Thus a significantly higher proportion of strong maternal impulses appeared in the woman having a flow of 6 to 8 days than in the group flowing from 2 to 4 days.

The findings have withstood the test of rigorous statistical analysis and have been confirmed in more recent studies. Aside from the work of Wiesner and Sheard[2] and more recently that of Riddle and his collaborators,[3] on the influence of prolactin on maternal behavior, I know of no data from the field of endocrinology which seem to throw light on the relationship. If your readers can offer further relevant data or explanation regarding the matter I shall be glad to receive the same.

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[1] LEVY, D. M.: Psychosomatic studies on some aspects of maternal behavior. *Psychosomatic Medicine* 4: 223. 1940.

[2] WIESNER, P. B., AND N. M. SHEARD: Maternal Behavior in the Rat. Oliver and Boyd, London, 1933.

[3] RIDDLE, O.: Lactogenic factor of the pituitary. *J. Am. Med. Assoc.* 104: 636. 1935.

CREATINE METABOLISM AND METHYL TESTOSTERONE

TO THE EDITOR:

I read with great interest the splendid article 'Influence of Methyl Testosterone on Muscular Work and Creatine Metabolism in Normal Young Men' in the November issue of the JOURNAL OF CLINICAL ENDOCRINOLOGY.[1]

CREATINE EXCRETION PER 24 HOURS IN HYPOGONAD MALES RECEIVING METHYL TESTOSTERONE

Case	Initial Values	Treatment with Methyl Testosterone					
		40 mg. or more daily				20 mg. daily	
	mg.	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*	*mg.*
P.W.	225	740	540			217	173
G.R.	80	400	460	750	840	125	100
J.H.	164, 36	320	360			169	
R.T.	38	510	440			120	
R.C.	0	266	300			80	110

I wish to report to you that similar findings were observed in our hypogonad cases (see table). We explained this phenomenon in terms of the recent works by Beard[2,3] on the effect of methylating substances on creatinuria. In view of this I believe that it may be suggested that large doses of methyl testosterone (40 mg. or more) act as methylating substances and that evidence of increased creatinuria probably represents a pharmacologic effect of an over-dose. You will note from our table that when dosage was lowered to a level of 20 mg. (quite sufficient for the desired physiologic effects) this excessive creatinuria disappeared.

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[1] SAMUELS, L. T., A. F. HENSCHEL AND A. KEYS: *J. Clin. Endocrinology* 2: 649. 1942.

[2] KELLY, C. J., AND H. H. BEARD: *J. Biol. Chem.* 133: 7. 1940.

[3] BEARD, H. H.: Annual Review of Biochemistry. Stanford Univ. Press, 1941.

Abstracts of CURRENT CLINICAL LITERATURE

Editor: DANIEL A. McGINTY. *Collaborators:* ISRAEL BRAM, JOHN C. BURCH, JOHN C. DONALDSON, MURRAY B. GORDON, R. B. GREENBLATT, E. C. HAMBLEN, CHARLES W. HOOKER, R. G. HOSKINS, J. E. HOWARD, J. P. PRATT, J. T. LEWIS, A. E. MEYER, C. A. PFEIFFER, BORIS B. RUBENSTEIN, PATRICIA H. SMITH, EMMERICH VON HAAM, HAROLD WOOSTER.

BOOK REVIEWS

CORNER, GEORGE W.

The hormones in human reproduction. Princeton University Press: Princeton, 1942.

This book is a clear and simple discussion of the rôle of the hormones in reproduction, which presupposes no special knowledge of biology. There is a brief discussion of the comparative physiology of reproduction. This is followed by accounts of the estrous and menstrual cycles. The discussions of estrogens, progesterone, and the androgens contain brief historical summaries of their discovery, and accounts of knowledge of their present status. An appendix gives an account of the nature of the sterol hormones.

KOCH, F. C., AND PHILIP E. SMITH.

Edited by Biological Symposia. Vol. IX. Jaques Cattell Press, Lancaster, 1942.

Two symposia are presented in this book. The first, "Sex hormones—their actions and metabolism," contains the following papers: The comparative biology of testicular and ovarian hormones, by Carl R. Moore; The comparative metabolic influences of the testicular and ovarian hormones, by A. T. Kenyon; The metabolism of estrogens, by E. A. Doisy; The excretion and metabolism of male sex hormones in health and disease by F. C. Koch. The symposium on "Hormonal factors in the inversion of sex" contains the following: Sex inversion in the plumage of birds, by C. H. Danforth; Sex inversion in the amphibia, by R. R. Humphrey; Hormonal factors in sex inversion: the effects of sex hormones on embryonic sexual structures of the rat, by R. R. Greene; Hormones and the experimental modification of sex in the opossum.—R. K. Burns, Jr.

ADRENALS

DORFMAN, R. I., B. N. HORWITT AND WM. R. FISH.

The presence of a cortin-like substance (cold protecting material) in the urine of normal men. *Science* 96: 496. 1942.

Ethylene dichloride extracts of pooled urines of normal men administered to adrenalectomized rats increase survival times when exposed to cold of 40–50%—approximately 6 hours for controls to 8 to 9 hours for treated rats. Cortin-like activity was not found in urines of patients with Addison's disease.—*D.A.M.*

HARKNESS, D. M., E. MUNTWYLER, F. R. MAUTZ AND R. C. MELLORS.

Electroyte and water exchange between skeletal muscle, "available (thiocyanate) fluid," and plasma in the dog following the administration of desoxycorticosterone acetate. *J. Lab. & Clin. Med.* 28: 307. 1942.

After a control period and a constant diet supplemented with 15 gms. of sodium chloride per day, ten dogs were given 1 mg. per kilo daily of doca for 14 days. As compared to the control series, there was a tendency for both plasma volume and "available (thiocyanate) fluid" to increase during the period of doca administration. At the end of the period of doca administration the plasma potassium and chloride concentrations had diminished while the sodium concentration increased. There was a similar loss of cellular potassium and increase of sodium in the muscle. There was no change in muscle chloride. Two weeks after discontinuing the drug the muscle potassium and sodium had not returned to normal levels. Despite the changes in electrolyte pattern, there was no striking change from normal in the relationship between intracellular and extracellular fluid.—*B.B.R.*

KOSTER, HARRY, AND LOUIS P. KASMAN.

Effect of desoxycorticosterone acetate in postoperative shock. *Arch. Surg.* 45: 272. 1942.

One hundred patients were selected alternately from 200 who had undergone surgery for conditions in which shock might reasonably be expected to develop during or after the operation. These persons were treated with desoxycorticosterone acetate and parenteral solution of sodium chloride but shock was neither prevented nor favorably influenced.—*Courtesy L. Reiner—Biol. Abst.*

PFIFFNER, J. J.

The adrenal cortical hormones. *Advances in Enzymology* 2: 325. 1942.

This is a review of the chemistry and physiology of the adrenal cortical hormones with particular emphasis on the number of hormones present in the gland. Intermediary metabolism of adrenal steroids is discussed.—*D.A.M.*

ENDOCRINE GENERAL

ALLEN, W. M., AND G. P. HECKEL.

The effect of progesterone in adolescent girls and young women with functional uterine bleeding. *Am. J. Obst. & Gynec.* 44: 984. 1942.

Results of treatment of 24 adolescent girls and young women with irregular uterine bleeding with progesterone or anhydro-hydroxyprogesterone are reported. It was observed in general that the administration of 30 mg. (5 mg. daily) of progesterone was followed by cessation of bleeding within 10 days of the last injection. Progesterone deprivation bleeding was described as occurring frequently. This is judged to explain bleeding which occurs the first few days after cessation of therapy. In about one-third of cases, cyclic bleeding occurred for 4 months or more after discontinuation of therapy whereas in about one-third of cases there were recurrences of abnormal bleeding in less than 4 months. In the other third of cases, there was amenorrhea for 2 to 3 cycles.—*E.C.H.*

FREED, S. C.

The present status of practical endocrine therapy. *Illinois M. J.* 82: 165. 1942.

Preparations of anterior pituitary are disappointing on the whole, with the exception of special high potency noncommercial growth hormone extracts in treatment of pituitary dwarfism. Chorionic gonadotropin is of definite value in the treatment of cryptorchidism where there is no anatomical barrier preventing descent of the testes. Chorionic gonadotropin is incapable of stimulating either the monkey or the human ovary. While ovulation in the human may be produced by administration of equine gonadotropin, there is no reason to believe that any but a normal woman, who ovulates spontaneously, will respond to this substance. Its use in sterility is questionable. Its use in ovarian dysfunctions is difficult to evaluate and impractical. There is some evidence that it may stimulate spermatogenesis. Its use in the treament of cryptorchidism is not as satisfactory as with chorionic gonadotropin. Diethylstilbestrol is highly active orally, and in dosages of 0.5 to 1 mg. a day will relieve the menopausal symptoms of a majority of patients. The main disadvantages are certain unpleasant symptoms in 10 to 20 per cent of cases. Estrogens are of the greatest value in the treatment of the menopause and complications of the menopause such as senile vaginitis and kruarosis. They are also of value in gonorrheal vaginitis of children. In the treatment of amenorrhea, estrogens may be administered to induce uterine bleeding. In the treatment of dysmenorrhea, estrogens have been used successfully to a limited extent. Such treatment is usually temporary and repeated courses of therapy may be necessary. Injection of estrogens in oil directly into the cervix results in practically immediate control of menorrhagia in women approaching the menopause. Diethylstilbestrol and estradiol compounds have been used for this purpose. By placing 25 to 50 mg. of estrogen in crystals or pellet form in a subcutaneous pocket, menopausal symptoms may be relieved for many months without supplementary therapy because of the retarded rate of absorption. Therapeutic use of progresterone has been disappointing. It is helpful in relieving premenstrual tension and is of possible value in the treatment of habitual abortion. There is evidence, that in many cases of habitual abortion the patient has a progesterone deficiency, and on this basis progesterone may be indicated. The most efficient androgen for therapeutic purposes is testosterone propionate. Testosterone is of value in the substitution therapy of eunuchoid or castrate men. The dosages required for satisfactory effects are from 60 to 150 mg. per week. The symptoms and signs of castration return with cessation of therapy. The androgen treatment of functional cryptorchidism has few advantages, nor has it any but psychic value in treatment of senility, psychic impotence or impotence due to other than

organic causes. It should not be used for treating disorders of sperm formation since it actually suppresses spermatogenesis. This substance has been used in certain ovarian dysfunctions such as dysmenorrhea, menorrhagia, painful breasts and premenstrual tension. The amounts required are rather large, 100 to 500 mg. per month. Adrenal cortex extracts are of value in the treatment of Addison's disease, although of doubtful value in borderline cases of adrenal insufficiency such as asthenia and toxemias. Desoxycosticosterone acetate, a synthetic steroid, is effective in the treatment of Addison's disease, but overdosage may result in hypertension or even heart failure. Its use in the treatment of the borderline cases of adrenal insufficiency is open to question. —*I.B.*

KOLLER, T. H.

Estrogens in urine and blood. *Helvet. Med. acta* 9: 392. 1942.

Estrogen concentration in the urine of pregnant women slowly increases until delivery and diminishes then rapidly. There is a certain correlation between the estrogen concentration in the serum of the mother just before delivery and the size of the placenta and of the newborn. Urinary estrogen concentration of non-pregnant women is as a rule rather low during menstruation and reaches several peaks during the intervals. In patients with dysmenorrhea, estrogen in the urine is often relatively high during menstruation, but there is no correlation between uterus contractility and urinary concentration.—*Courtesy, E. Fischer—Biol. Abst.*

LISSER, H., AND ROBERTO F. ESCAMILLA.

Testosterone compounds in the male. Clinical indications and methods of administration. *Urol. & Cutan. Rev.* 46: 87. 1942.

Synthetic testosterone compounds are effective parenterally, by implantation, by inunction, orally and sublingually. The relative advantages of the several modes of administration are discussed and comparative dosages are given. The indications for testosterone therapy in the male are listed and discussed.—*Author's Summary.*

PINCUS, GREGORY, AND W. H. PEARLMAN.

Metabolism of estrone in men and non-pregnant women. *Endocrinology* 31: 507. 1942.

The estrogen titers of 3 principal phenolic fractions of the pooled urines of 8 male and 13 female patients were determined before and after injection of estrone. The fractions were (a) the 'strong' phenolic, (b) the 'weak' phenolic non-ketonic and (c) the 'weak' phenolic ketonic. By the application of such fractions of certain partitionings between solvents and an alcoholic separation (succinic anhydride), evidence was obtained that the 3 principal urine fractions behave as though they contain estriol, estradiol, and estrone, respectively. After the injection of estrone the titer of all 3 fractions increased. The calculated recoveries indicate a conversion of the injected estrone principally to estriol, but also to estradiol. Less than 2% of the injected estrogen could be accounted for by the increased urinary excretion. Although the female patients have higher preinjection urinary titers than the male patients, the calculated recoveries of injected estrogens show no marked difference between the sexes.—*Author's Summary.*

ROCK, J., AND A. T. HERTIG.

Some aspects of early human development. *Am. J. Obst. & Gynec.* 44: 973, 1942.

Data on 12 early conceptuses obtained from 12 of 60 excised uteri in the course of a systematic search for early ova were analyzed with particular regard to: 1) the probable time of ovulation as evidenced by embryo age and endometrial histology; 2) the time of nidation; 3) the location of embedment; and 4) the frequency of abnormal ova. The ages of the embryos varied from 7 to 8 days to 16 to 17 days. From comparison of the age of the embryo with associated endometrial histology, it was apparent in two well controlled cases that ovulation occurred about 14 (13 to 15) days before anticipated menstruation. From studies of three embryos, it was concluded that nidation may take place at a variable age of the embryo (from 5th to 8th day of age) and on an endometrium which may vary in phase from the 19th to the 22nd day of a 27-day cycle. The seven normal conceptuses were found in the posterior wall of the uterus and the five abnormal ones of the anterior wall. Five (42 per cent) of the twelve embryos were so pathologic as to indicate probable early abortion.—*E.C.H.*

SNYDER, F. F.

The experimental production of toxemia of pregnancy. *Am. J. Obst. & Gynec.* 44: 1091. 1942.

At the 25th day of pregnancy 34 rabbits were given an injection of chorionic gonadotropin, (dosage ranging from 10 to 144 r.u.) designed to

induce ovulation. Uteroplacental apoplexy involving premature separation of placenta was observed following this hormonal induction of ovulation.—*E.C.H.*

TWOMBLY, G. H., AND H. C. TAYLOR.

Inactivation and conversion of estrogens *in vitro* by liver and other tissues from human cancer patients and from mice of strains susceptible to mammary carcinoma. *Cancer Research* 2: 811. 1942.

When slices of liver were immersed in a watery solution of estradiol, the estrogenic activity of the hormone was diminished or destroyed. This was particularly noticeable when rat liver was used. Human liver slices did not appear to have as great a capacity for inactivated estradiol as did rat or mouse liver slices. This difference may explain why estrogens are detectable normally in human urine and not in the urine of the rat and the mouse. Tests run with livers from normal as compared with cancerous patients failed to show any consistent difference in estradiol destroying capacity. The same thing was true of the livers of 4 strains of mice differing in their susceptibility to mammary cancer.

Stronger solutions of estrone, when mixed with pregnant rabbit uterus increased their activity in such a way as to suggest that the estrone quantitatively is turned into estradiol. When malignant human tissues were compared with similar normal human tissues, this converting ability was present in only a few of both types. However, the tendency to destroy the activity of estrone by such a procedure seems to be more marked in the normal than in the cancerous tissue.—*Author's Summary.*

ZURROW, H., G. SOLAND, C. KLEIN AND S. GOLDMAN.

The effect of testosterone propionate in the treatment of arteriosclerosis obliterans. *J. Lab. & Clin. Med.* 28: 269. 1942.

Twenty-three patients suffering from arteriosclerosis obliterans were treated with biweekly muscular injections of 25 mg. of testosterone propionate. Fifteen other patients were used as controls and received biweekly intramuscular injections of 3 cc. normal saline. On the basis of author's criteria there were no significant differences between the treated and control cases with regard to the amplitude of blood vessel pulsation, arterial calcification, skin or muscle atrophy, pain while at rest, or claudication. Thus at the end of a 3–7 month period of treatment, an 8–14 month period, and a 14–18 month period, there were no changes in functional capacity or reserve, or in anatomic conditions, as a result of treatment with testosterone propionate.—*B.B.R.*

GONADS

BREWER, J. I.

Studies of the human corpus luteum. Evidence for the early onset of regression of the corpus luteum of menstruation. *Am. J. Obst. & Gynec.* 44: 1048. 1942.

Ninety-seven specimens from patients undergoing pelvic operations were studied. Definite regressive changes were found to appear approximately at the 8th to 10th day of the corpus luteum cycle. The regressive changes were described as follows: 1) A marked decrease in the amount of blood in the vessels of the granulosa lutein layer and regressive changes in the vascular system. 2) Fatty degeneration and degeneration by simple atrophy of the granulosa lutein cells. 3) Increase in the amount of visible lipids in the granulosa lutein cells. 4) A sharp increase in cholesterol esters and a gradual diminution of the phospholipin content, as determined chemically. 5) Regressive changes in the endometrium. 6) A decrease in the amount of pregnanediol excreted in the urine, as determined by other workers.—*E.C.H.*

CARTER, B.

A bacteriologic study of pyometra. *Am. J. Obst. & Gynec.* 44: 1074. 1942.

Endocrine interest in this article lies in reference to production of pyometra in experimental animals by injections of estrogens and in the statement that anaerobic organisms capable of invading the cervix and uterine cavity are found normally in the vaginas of postmenopausal women in a higher incidence than in the vaginas of childbearing women.—*E.C.H.*

CHUTE, R., AND A. T. WILLETTS.

Treatment of cancer of the prostate with castration and administration of estrogen. *New England J. Med.* 227: 863. 1942.

Thirty-seven cases of inoperable prostatic carcinoma were treated by castration alone (2 cases), diethylstilbestrol alone (8 cases) or by combinations of the two (27 cases). General results were satisfactory, only 1 patient failing to show any improvement. Results from stilbestrol therapy alone were equal to combinations of castration and stilbestrol although improvement

lasted only during the period of treatment. Stilbestrol accelerates the effects of castration. Results included rapid, effective and lasting relief from metastatic pain, better appetite, improvement in weight and strength, feeling of well-being and reduction and softening of the prostate. In 9 of 13 cases with inability to urinate retention so improved as to urinate readily. 17-ketosteroid excretion was reduced after castration. Bony metastases, demonstrable by X-ray, progressed despite treatment. Unpleasant side-effects from stilbestrol disappeared on reduction of dosage or discontinuance of treatment.—*D.A.M.*

FELDMAN, S., J. POLLOCK AND A. R. ABARBANEL.

Treatment of senile pruritis with androgens and estrogens. *Arch. Derm. and Syph.* 46: 112. 1942.

Twelve of 16 patients showed uniformly good results when treatment is continuous with sex specific hormone. Dosage varies with individual patients, 10 mg. of testosterone propionate or 1 mg. of estradiol dipropionate constituting an average weekly dose. In females undesirable side effects (uterine bleeding, painful nipples) are readily counteracted by combining estrogen with androgen.—*D.A.M.*

GOLDBERG, MINNIE B., AND H. LISSER.

Hypogonadism in acromegaly. Report of two cases, with improvement from male and female sex hormone. *Clinics* 1: 644. 1942.

Case 1: Was that of a young man 29 years of age with relatively early and mild acromegaly. He responded satisfactorily to two courses of pituitary irradiation except in respect to hypogonadism which was characterized by diminution in size of the genitalia, loss of libido and potentia; development of a peculiar sallow-yellow discoloration of the skin, markedly diminished beard growth, and a noticeable lack of vigor and strength. Testosterone propionate 25 mg. parenterally three times a week for twelve weeks resulted in return of sexual power, restoration of normal beard growth, strength and vigor, and disappearance of the fawn color of the skin. By the combination of X-ray and specific sex hormone therapy this young married man was completely rehabilitated and normalized.

Case 2: Was one of long-standing acromegaly (24 years) in a 44 year old unmarried woman. As a result of several surgical interventions and five courses of pituitary irradiation her acromegaly became relatively quiescent. Amenorrhea, one of the earliest symptoms in female acromegalics, began at the age of 20 years and had persisted for 22 years. Breasts and uterus were atrophic and the ovaries felt small and hard. She had suffered from recurrent labial herpetic ulcers. For many years she had been nervous, restless and cyclically melancholic. Under cyclical stilbestrol therapy (42 mg. per month) regular menstruation was restored (after 22 years amenorrhea), the labial ulcers vanished and the menopause-like symptoms greatly relieved.—*Author's Summary.*

GREENE, L. F., AND H. E. ESSEX.

Effects of drugs on ureteral activity. *Proc. Staff Meet. Mayo Clin.* 17: 404. 1942.

Hydrophorograph records of ureteral peristaltic activity of dogs anesthetized with barbiturates, ether and chlorolose were obtained following intravenous administration of various drugs. Epinephrine and mecholyl increase ureteral tone and peristaltic rate. Ergotoxine produced relaxation in tone and decrease in amplitude of contraction. Atropine produced inconsistent results and not in harmony with those of previous workers.—*D.A.M.*

HERBST, WM. P.

The effects of estradiol dipropionate and diethylstilbestrol on malignant prostatic tissue. *Tr. Am. A. Genito-Urin. Surgeons* 34: 195. 1941.

Seven patients with carcinoma of the prostate were treated by the administration of estradiol dipropionate or diethylstilbestrol. One mg. doses were administered, parenterally one to three times weekly for varying periods. Patients with severe pain due to osseous metastasis were almost immediately relieved of the necessity of taking opiates. There was noted a definite decrease in the total bulk of the malignant mass. In three instances where benign tissue was examined histologically after the administration of these substances, no changes could be recognized which would indicate that anything characteristic of administration of estrogen was present.

No toxic manifestations were noted except a little edema in one patient who was feeble and under treatment for hyperplasia. These symptoms cleared up with a reduction of the dose to ½ mg.

Breast reactions were noted in some but not all cases and consisted of tenderness and enlargement of the breast tissues.—*D.A.M.*

IVY, A. C.

The functional anatomy of labor, with especial reference to the human being. *Am. J. Obst. & Gynec.* 44: 952. 1942.

In this article, the author correlates the course of the wave of contraction with the morphological pattern of the uterine musculature in the human being, summarizes the evidence regarding the growth of the isthmus uteri and its unfolding and inclusion as a part of the general uterine cavity during pregnancy in the human being and monkey and reports certain observations resulting from measurements made on all the frozen sections of the human uterus reported in the literature. Endocrinology is not specifically discussed.—*E.C.H.*

LISSER, H., A. PALMER, AND D. G. MORTON.

Arrhenoblastoma—Removal followed by refeminization and pregnancy. *Clinics* 1: 768. 1942.

A 25 year old married housewife whose menses had been normal previously ceased menstruating one year before appearance at the clinic. During this year of amenorrhea, an heterosexual hypertrichosis appeared together with hypertrophy of the clitoris. The right ovary was palpated as a freely movable cystic mass about 8 cm. in diameter. X-rays of skull, cervical spine and pelvis all showed a very slight osteoporosis. Blood pressure and glucose tolerance were normal. The sella turcica seemed normal and intravenous pyelograms yielded no evidence of an adrenal tumor. Diagnosis of arrhenoblastoma was verified surgically and pathologically. The cytological structures of the tumor varied from sarcomatoid to that of a testicular adenoma. Menstruation was restored 28 days after removal of the tumor and occurred regularly thereafter. Ten months later a positive Friedman test confirmed the suspicion of pregnancy. The abnormal hair of her face and body was of a softer texture and much of it had fallen out. The clitoris had atrophied to half of its former size.—*Author's Summary.*

MARQUARDT, C. R., AND W. A. FLAHERTY.

Carcinoma of prostate gland with special reference to endocrine treatment. *Urol. and Cutan. Rev.* 46: 343. 1942.

In forty-six patients with prostatic carcinoma an attempt was made to secure androgen suppression either by castration or administration of diethylstilbestrol. The majority of patients presented X-ray or clinical evidence of dissemination of prostatic growth with periprostatic involvement. Thirty-one were castrated and fifteen treated with diethylstilbestrol. Six patients entirely unimproved by castration were later given diethylstilbestrol without benefit. Likewise, six diethylstilbestrol refractory patients were castrated without improvement.

During nineteen months there have been thirteen deaths, a mortality of 30.5%. Two patients have died from pulmonary embolism, two from cardiovascular failure, one from bowel obstruction and seven from pyelonephritis. One patient with hypertension died six months after castration from apoplexy. This patient had marked clinical improvement following castration and had X-ray evidence of improvement in his metastatic bone lesions.

Sixty per cent of all patients treated showed clinical improvement as evidenced by relief of urinary obstruction and pain, gain in weight and improved blood picture. The prostate as palpated per rectum has decreased in size and became noticeably softer.—*Author's Summary.*

ROBSON, J. M., AND A. SCHÖNBERG.

A new synthetic estrogen with prolonged action when given orally. *Nature, London* 150: 22. 1942.

$\alpha\alpha$-di(p-ethoxyphenyl) β-phenyl bromoethylene in oil when injected subcutaneously in 4 doses over 48 hours into ovariectomized mice or when administered orally in single doses exerts a much more prolonged estogenic action than diethylstilbestrol, estradiol dipropionate, triphenyl chlorethylene or other estrogenic substances.—*D.A.M.*

WINN, W. C., AND H. HUDNALL WARE.

Hydatidiform mole. *Virginia M. Monthly* 69: 678. 1942.

In several cases of hydatidiform mole, the spinal fluid gave a negative test for gonadotropin by the rabbit ovulation test, although the urine was positive. Positive rabbit ovulation tests with urine were obtained as long as 4 and 7 weeks postoperatively in 2 patients.—*Courtesy R. J. Main.—Biol. Abst.*

HYPOPHYSIS

ESCAMILLA, ROBERTO F., AND H. LISSER.

Testosterone therapy in a male case of hypophyseal cachexia (Simmonds' disease). *Clinics* 1: 710. 1942.

Testosterone therapy had a decidedly beneficial effect in a characteristic case of Simmond's disease caused by a craniopharyngioma. Partial removal and drainage of the Rathke's pouch cyst relieved increased intracranial pressure but did not favorably influence pituitary function. Six months later, at the age of 20 years, testosterone therapy was started and during the subsequent 19 months of treatment, this young man grew 1¾ inches and gained 23¼ pounds. Prior to treatment his height was 5 feet 4¾ inches (164 cm.) and his weight 67 pounds (30.5 kg.). Normal sexual function appeared and the genitalia enlarged considerably. The secondary sexual characteristics improved also. The patient's general strength and vigor were strikingly benefited.

It is significant that the excellent result achieved represents the satisfactory use of a potent hormone not derived from the endocrine organ primarily responsible for the endocrinopathy—namely, testosterone as an aid in combatting a severe panhypopituitarism.—*Author's Summary.*

ROCHAT, R. L.

Simmonds' disease and amenorrhea following fetal hypophyseal grafts. *Helvet. med. acta* 9: 373. 1942.

A 23 year old girl with Simmonds' disease (pituitary cachexia) was considerably improved by intramuscular implantation of a fetal hypophysis. The patient increased in weight during 4 months from 42 to 51 kg., her basal metabolism returned to normal values, and the first menstruation occurred after 6½ years of complete amenorrhea.—*Courtesy, E. Fischer—Biol. Abst.*

PANCREAS

JOSLIN, E. P.

The use of insulin in its various forms in the treatment of diabetes. *Bull. New York Acad. Med.* 18: 200. 1942.

A review of the forms of insulin in use today in treatment of diabetes, with reasons for preferential use of certain forms.—*D.A.M.*

PARATHYROID

ROSS, WM. F., AND THOMAS R. WOOD.

The partial purification and some observations on the nature of the parathyroid hormone. *J. Biol. Chem.* 146: 49. 1942.

A method is described for the preparation of parathyrod extracts of approximately 3 times the activity of any hitherto reported. Present concepts regarding the protein nature of the active principle are reinforced by a study of its pepsin digestion under suboptimal conditions, by its ultraviolet absorption spectrum, and by its stability to electrodialysis. Ultracentrifugal study of the preparation shows it to be heterogeneous, consisting of at least 2 components, 1 of molecular weight of roughly 20,000 and another of 500,000 to 1,000,000. There is some reason to believe that the activity may be associated with the low molecular material.—*Author's Summary.*

THYROID

BARTLETT, WILLARD.

Essential biochemical derangements in hyperthyroidism. *Arch. Surg.* 45: 103. 1942.

The correlation between thyrotoxicosis and acidosis was studied. The carbon dioxide-combining power of the plasma of 18 patients who were resting and whose physiological needs for water and nourishment were met, was maintained within normal limits even in severe exacerbations, but on exertion a prompt decrease in alkali reserve occurred. In 8 of 18 patients who showed prompt improvement after thyroidectomy a measurable rise in alkali reserve was observed. During improvement on treatment preliminary to thyroidectomy and subsequent to operation, the total titratable acid of the urine, of the organic acids and of ammonia fell considerably and the pH of the urine rose. The patient's intake of food was kept constant quantitatively and qualitatively. The bearing of these findings on the pathology and symptomatology of hyperthyroidism was discussed.—*Courtesy L. Reiner.—Biol. Abst.*

HIMWICH, H. E., C. DALY, J. F. FAZEKAS AND H. C. HERRLICH.

Effect of thyroid medication on brain metabolism of cretins. *Am. J. Psychiat.* 98: 489. 1942.

The brain metabolism of 11 cretins ranging in age from 9 to 31 years was studied before and after administration of desiccated thyroid. Alterations of brain metabolism were determined by taking into consideration the changes of the arterio-venous O_2 difference and cerebral blood flow which was estimated with the aid of a thermostromuhr inserted in the internal jugular vein. Blood was collected from an artery and the internal jugular vein and analyzed for O_2. The average of 29 arterio-venous O_2 differences of 11

cretins was 5.74 vol. % before thyroid administration and that value was reduced to 4.69 vols. % on 25 observations on 9 patients after therapy. In 8 cretins the average acceleration of cerebral blood flow was 57%. An average increase of 32% in cerebral metabolism was thus revealed as a result of the administration of thyroid. This increase in cerebral metabolism afforded a basis for alterations in the cerebral electrical potentials and psychological reactions of these patients. The electroencephalograms disclosed a rise of the energy level in certain frequency ranges. Psychologically, improvement was either absent or of questionable significance in any of the higher processes. The greatest change was that of an acceleration of psychological activity, an acceleration made possible by the increased energy expenditures stimulated by thyroid medication. —*Courtesy B. L. Pacella—Biol. Abst.*

KING, EARL J., AND C. G. BARNES.

Galactose tolerance in thyrotoxicosis. *J. Path. and Bact.* 54: 526. 1942.

Less than 8% of normal subjects have a galactose index over 120. In 37 thyrotoxic patients the index was over 120 in all except 4 very mild cases. (The galactose index is the sum of the blood galactose values in mg. at ½, 1, 1½ and 2 hours after the injection of 40 g. of galactose.)—*Author's Summary.*

MAN, E. B., A. E. SMIRNOW, E. F. GILDEA AND J. P. PETERS.

Serum iodine fractions in hyperthyroidism. *J. Clin. Invest.* 21: 773. 1942.

Protein-bound iodine was separated from inorganic iodine in serum by precipitating with the Somogyi reagent used for preparation of blood filtrates. Iodine in the precipitate and filtrate was determined by the permanganate acid ashing technique. When solutions of diiodotyrosine or thyroxin were added to serum the precipitate from serum contained at least 80% of diiodotyrosine iodine and virtually all thyroxin iodine.

The precipitable iodines of 6 normal subjects given 10 to 45 drops of Lugol's solution daily for 1 to 7 days agreed within 1.6 gamma per cent with serum total iodines after Lugol's administration was stopped. In normal individuals after cessation of iodine administration, 2 days, but occasionally 4 to 10 days, were necessary for elimination of excess inorganic iodine from the serum.

Precipitable iodines were studied in 15 hyperthyroid patients whose clinical diagnosis was confirmed by pathological study of the glands. Before treatment with iodine, serum total or precipitable iodines were between 9.4 and 33.7 gamma per cent per 100 cc., distinctly above the normal range of 4 to 8 gamma per cent. In 5 patients, before administration of iodine, total and precipitable iodines agreed within 2.0%. After Lugol's, before thyroidectomy, precipitable iodines of 11 of 14 patients decreased to about 8.0 gamma per cent. In 3 patients, whose precipitable iodines did not decrease noticeably, clinical response to iodine administration was poor. If basal metabolisms are unsatisfactory, the behavior of the precipitable iodine is of diagnostic significance.

Serum total or precipitable iodines of 10 of 15 patients were within or just above the normal range about 2 weeks after thyroidectomy.—*Author's Summary.*

PFEIFFER, D. B., AND A. G. MARTIN.

Diagnosis and surgical treatment of goiter. *Med. Clin. N. America* 26: 1739. 1942.

This is a brief discourse on the diagnosis and treatment of the common types of thyroid enlargements from the surgical viewpoint, with illustrative case histories. The conditions emphasized are exophthalmic goiter, toxic and nontoxic adenomas, and thyroiditis. The possible harmful effect of iodine administration is stressed. The general methods of diagnosis, preparation, and postoperative care for colloid goiter, diffuse toxic goiter, nodular goiter, both toxic and nontoxic, and thyroiditis are discussed. At the present time, definitive treatment of the great majority of thyroid disorders means surgical treatment. However, there is more to the treatment of thyroid disease than operation alone. Cardiac, nutritional and endocrine factors call for close coöperation between internist and surgeon throughout the treatment of thyroid disease.—*I.B.*

PICARDO, T., AND G. NARANJO.

Treatment of hyperthyroidism. *Rev. Med. (San Jose)* 5(96): 175. 1942.

The authors describe a new treatment for hyperthyroidism. It consists in giving subcutaneous injections of beef thyroid peptone (5%) over a period of months. They cite one case in which a total of 50 cc. (2.5 gm. peptone) was given for

4 months. The patient's pulse rate was lowered, she gained weight and the exophthalmos was definitely reduced. This type of treatment has several advantages over previous treatments. The extract is not antigenic. It acts specifically against the thyrotropic hormone, and does not neutralize the effect of other hypophyseal stimuli. Repeated injections do not lead to hypertrophy of the thyroid.—*Courtesy E. McNeil, Biol. Abst.*

ROSE, E.

The diagnosis and treatment of thyroid disease. *Med. Clin. N. America* 26: 1711. 1942.

Hypothyroidism often masquerades in a variety of clinical disguises. The optimal dose of desiccated thyroid varies from patient to patient. The same brand of thyroid substance should be used throughout the course of any given case. The treatment of hypothyroidism should not be abandoned because of the appearance of signs of overdosage. Hyperthyroidism often simulates other diseases and other diseases may simulate hyperthyroidism. Subtotal thyroidectomy competently performed after proper preparation is the best single method of treating hyperthyroidism. Irradiation is useful in carefully selected cases. The beneficial effect of iodine in hyperthyroidism is usually temporary. The principal use of this drug should be in the preparation of patients for thyroidectomy. Nontoxic diffuse goiter seldom responds completely to iodine therapy, but such therapy is justifiable because of its occasional good results and possible prophylactic effect. Nontoxic nodular goiters should be treated surgically unless special contraindications exist.—*Author's Abst.—I.B.*

ROSENKRANTZ, J. A., MAURICE BRUGER AND A. J. LOCKHART.

Studies on galactose tolerance with especial reference to thyroid disease. *Am. J. M. Sci.* 204: 36. 1942.

Age alone has no influence on the galactose tolerance curve. Impaired galactose tolerance occurred in hyperthyroidism, Bright's disease, upper respiratory infections, malignancy, and as a concomitant finding of sulfonamide therapy. The impairment was most marked in thyrotoxicosis. Diabetic patients have a normal tolerance. —*C.P.*

SCHMIDT, C. R., A. E. HERTZLER.

Cardiotoxic goiter—a distinct entity. A preliminary report. *Endocrinology* 31: 684. 1942.

The present day concept that chronic hyperthyroidism is due to excessive activity of normal glandular tissue fails to explain the normal or slightly elevated basal metabolic rate in the majority of patients with goiters of long duration. Three types of experimental evidence suggest that goiters produce a perverted rather than an excessive secretion. The colloid from degenerated and nodular goiters associated with cardiac symptoms show changes in the staining properties of the colloid: with hematoxylin-eosin certain follicles stain basophilic instead of acidophilic; with Mallory's aniline blue dye these areas stain orange instead of the usual deep blue. Chemical analysis of thyroglobulin reveal that a goiter is incapable of both storing and synthesizing thyroxine in normal amounts. Extract from toxic goiters contains a principle which, when fed to thyroidectomized rats, causes an increased heart rate without a corresponding increase in O_2 consumption.—*Author's Summary.*

SEARLE, H. H.

Thyroid disease. Experiences and conclusions of a thyroid committee. *California & West. Med.* 57: 184. 1942.

The nodular goiter presents a dangerous threat of malignant degeneration and therefore demands removal. Toxic diffuse goiter may be treated by partial ablation either surgically or by X-ray. Iodine should be used in toxic goiter only before surgery. Resection should be more radical in children than in adults. In experienced hands, mortality rate in thyroid surgery approaches zero and complications are rare.—*M. L. Ilsley (Biol. Absts.).*

SOLEY, M. H.

Exophthalmos in patients with various types of goiter. *Arch. Int. Med.* 70: 206. 1942.

In nontoxic nodular goiter the eyes of patients are not more prominent than those of normal persons (Hertel exophthalmometer). Patients with toxic nodular goiter tend to have more prominent eyes than normal persons but not so prominent as in patients with toxic diffuse goiter. The eyes of patients with toxic diffuse goiter are significantly more prominent than those of normal persons. Exophthalmos becomes significantly greater following subtotal thyroidectomy in over 50% of patients with toxic diffuse goiter. At the same time the majority lose the stare associated with hyperthyroidism. If patients with toxic diffuse goiter receive X-ray treatment, the progression of exophthalmos occurs to a less degree and less frequently than in the group in whom a subtotal thyroidectomy is done.—*Author's Abst.*